THE DEVELOPMENT OF THE PRONEPHROS DURING THE EMBRYONIC AND EARLY LARVAL LIFE OF THE CATFISH (ICTALURUS PUNCTATUS)

AN ESSAY

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INTRODUCTION

Anatomists and embryologists are almost universally agreed that the excretory function in adult Teleosts is performed by organs of a higher degree of specialization and of later ontogenetic development than the pronephros. The functional organ is usually accorded the value of a mesonephros, or, in some cases, of a metanephros. The majority of Teleosts of which we have any description in the literature, however, possess in the adult a structure usually referred to as the "head-kidney",(1) situated anterior to the air-bladder at a level adjacent to the basi-occipital bone, or the first cervical vertebra. The presence of this organ in the location occupied in the embryo by the pronephros has led to much speculation as to what relation, if any, exists between the two organs.

The peculiar condition of the head-kidney of the catfish

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(1) Although the terms "head-kidney" and "pronephros" are frequently used synonomously in the literature, especially in works dealing with the more generalized aspects of the subject, a distinction is considered necessary to avoid ambiguity. The term "head-kidney" is believed to be of value merely as an anatomical term denoting a structure with certain specific topographical relations in the adult. "Pronephros" is used to apply only to the embryonic excretory organ consisting of tubule, glomus, and pronephric chamber. Where these tissues are retained in functional state in the adult, the term "pronephros" may be applied. This view has the support of Grosglik and of Felix.

was called to the author's attention by Dr. R. P. Cowles, who suggested that a study of the embryological development of the excretory organs might be expected to furnish evidence as to the nature of the adult head-kidney. The present investigation was therefore undertaken with the intention of providing an account of the pronephros in the embryonic and early larval stages of the catfish, which might serve as a basis for a future study of the structural changes through which the pronephros of the young fish undergoes transformation into an organ the external characteristics of which are distinctly non-renal.

Acquaintance with the literature dealing with the problem immediately reveals the fact that the greatest possible diversity of opinion exists upon the morphological and physiological value of the head-kidney. It also becomes evident that early investigations were based largely upon superficial observation and inexact methods. Their interest is accordingly little else than historical. An extensive and accurate investigation of the whole subject therefore remains a desideratum.

A survey of the fate of the embryonic pronephros must include several questions. Does the pronephros ever persist in the adult Teleost as a functional renal organ? Is the structure commonly referred to in the literature as the "head-kidney" to be considered as derived from the embryonic pronephros, -the embryonic organ having undergone modification in structure and function? If not, are we to accept the view that the pronephros has completely disappeared, and that an organ of independent origin has arisen in the adult, its topographical relation to the remainder of the organism corresponding to that of the pronephros in the embryo? These questions will be considered in the discussion to follow.

HISTORICAL SUMMARY

Persistence of the pronephros as a functional renal organ

Hyrtl (1851), in a study of the excretory apparatus of some two hundred species of Teleosts, says, "in many species we find the head-kidney only, -the body kidney is lacking." Among the forms which Hyrtl describes as having a functional pronephros without other excretory organs are several members of the group Pediculati, (called by him "Pectorales Pediculati"), Lophius piscatorius, Chironectes punctatus, and Antennarius marmoratus; Pterois volitans, and "most genera of Gymnodontes and Sclerodermi".

Hypostomus verres (Plecostomus) is also described as having flat, triangular kidneys which extend over the first vertebra, and "are therefore head-kidneys only."

Hyrtl's interpretation of his findings in Lophius is disputed by Balfour, (1882-a), who considers the organ in question to be merely the "ordinary Teleostean kidney" which has become shifted anteriad because of other modifications of form in this highly specialized fish. Although subsequent discussion will show that the phrase "ordinary Teleostean kidney" is of no significance, it may be inferred from the context that Balfour attributes to the renal organ of Lophius the morphological value of a mesonephros. This he considers a more reasonable assumption than to postulate the loss of the "ordinary" excretory organ of Teleosts, and the retention of a larval organ which atrophies in less specialized Teleosts.

Calderwood (1891-92) declines to accept the theory of Balfour that the kidney of Lophius represents a mesonephros shifted forward from its normal position. Such a displacement he considers a structural impossibility. From his findings in Dactylopterus volitans and Cyclopterus lumpus he argues that "in the developing embryo, the segmental duct and pronephros are developed at a much earlier period than the mesonephros, and must be permanently separated from all abdominal viscera before the completion of the mesonephros." A second objection is constituted by the fact that in the forms studied by Calderwood the pronephros is encased in bone, so that if the body kidney grew forward to encroach upon the pronephros, the former would have to "penetrate the air-bladder and the scapular notch." Calderwood implies, at least, acceptance of the view of Hyrtl, for he states: "in adult Teleosteans the renal function is performed in some cases - - by the head-kidney only." Little value can be attached to the opinion of Calderwood in this instance, since it is based upon observation, not of Lophius, but of an entirely unrelated form.

An intermingling of pronephric and mesonephric elements appears entirely possible to Emery (1882). In the larvae of some forms (Blennius) the pronephros is in structural continuity with the mesonephros. Thus, "ce que l'on appelle chez l'adulte rein céphalique ne correspond pas toujours exactement au pronéphros de l'embryon, puisqu'il peut contenir en outre des éléments du pronéphros des tubes et des glomérules du mesonephros." He suggests that the massive "head-kidney", rich in uriniferous tubules, which occurs in Merlucius esculentus and in Lophius may be a structure of this sort.

Audigé's (1910) observations on the gross morphology and histological structure of the kidney of Lophius led him to adopt the view that it is unquestionably a mesonephros.

The embryological data which might be expected to offer the most convincing solution of the problem is regrettably lacking. It has been pointed out, however, that no available evidence lends support to the view that the excretory organ of Lophius is a pronephros. The occurrence in an adult Teleost of a pronephros without other excretory organs would, if established, constitute a unique instance within the group, and indeed among Vertebrates as a whole.

The question of the coexistence of a functional pronephros and mesonephros, however, forms the subject of a controversy which originated over half a century ago.

The pronephros of Fierasfer is claimed by Emery (1880) to persist in a functional state in the adult together with a comparatively rudimentary mesonephros. (Note: The genus Fierasfer has developed a curious relation, neither strictly parasitic nor commensal, to certain Holothurians, in the interior of which it passes the greater part of its existence as a "lodger". (Boulenger, 1904) In his monograph on "Le Specie del Genere Fierasfer nel Golfo di Napoli", Emery described the anterior region of the kidney of the adult as containing a large glomerulus (2) which is definitely related to the anterior end of the excretory ducts. "La porzione anteriore dilatata di ciascun rene contiene un solo voluminoso glomerulo do Malpighi con un tubulo renale aggomitolato." This occurrence of the "giant glomerulus", so characteristic of the Teleost pronephros and its morphological continuity with the kidney duct constitute, according to Guitel (1906), the only valid criterion by which a structure may be declared a functional pronephros or the homologue(3) of a pronephros. By the term "adult", according to general

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(2) Although the majority of embryological papers of a research character use both glomus and glomerulus interchangeably in referring to the large vascular organ of the pronephros, in works of a more general nature only the term glomus is used in referring to this structure. Kerr and Kingsley, however, employ the term "glomus" only when several glomerular strands of successive somites have coalesced into a single mass. Apart from the reservations of these authors, there seems to be no essential distinction in meaning, and for the sake of convenience, therefore, the term glomus will be used (in the part of the paper dealing with the original investigation) to designate the structure found in the pronephros, and glomerulus will be reserved for the Malpighian glomerulus of the mesonephros. In the section on the literature, however, the terms will be used interchangeably in the manner of the particular author under discussion.

(3) The word "homology" is employed in a peculiar sense in the literature on the Teleostean pronephros. The majority of papers reviewed refer to the embryonic pronephros as "homologous with" the adult head-kidney, although they undoubtedly mean merely that the former has become transformed into the latter. The term as used in the succeeding discussion of the literature will have this unusual significance, except in a few instances where its ordinary connotation will be apparent.

usage (Audigé, etc.), we understand an animal which has attained sexual maturity. If Emery had this restriction in mind when he used the term "adult", the morphological continuity of pronephric glomerulus and ureter which he describes indicates the probability that we have here an established case of a functional pronephros in the adult. A similar condition is later described (Emery, 1881-82) for young *Atherina* and *Mugil*, and for embryos of *Zoarces*. Here a compact pronephros occurs, containing convoluted tubules and a single large glomerulus which derives its arterial supply directly from the aorta.

These relations in the larvae, Emery insists, are identical with those in the adult *Fierasfer*. In spite of the lack of a complete embryological series he feels justified in asserting that the embryonic pronephros may remain active in the adult. The essence of his contention is "dass die embryonale Vorniere bei erwachsenen Teleostiern fortbestehen kann, und in vielen Fällen wirklich fortbesteht."

This condition, Emery states, (1882), obtains also for the adult *Zoarces*: "J'ai trouvé, dans le *Zoarces* adult, le glomérule unique du pronéphros parfaitement conservé et en continuité avec le conduit rénal; en d'autres termes, il y a là un veritable pronéphros larvaire."

The presence of a functional pronephros in young specimens of *Zoarces*, *Mugil* and *Atherina* is of no significance for the present discussion, but the contention of Emery that this organ occurs in adult Fierasfer and Zoarces is of considerable historical importance, as the first well-substantiated instance of this phenomenon.

Those who criticize Emery, however, contend that his proofs are not convincing. A study of four Teleosts (Cyprinus carpio, Esox lucius, Rhodeus amarus, Gasterosteus aculeatus) made by Grosglik (1885) demonstrated that in these forms the degeneration of the pronephros occurs late. Although the first changes are initiated soon after the formation of the mesonephros and the fuller development of other organs, these occur in the posterior region of the head-kidney, the anterior, glomerular region remaining unchanged until sexual maturity is attained. Tubules and glomerulus then are absent only in completely adult individuals. On the basis of these observations, Grosglik assumes that the pronephros of all Teleosts is reduced in a similar manner, and that Emery's claim for the persistence of a pronephros in adult Fierasfer and Zoarces are "without doubt" based upon observations of specimens not fully grown. In this view, Grosglik is supported by Audigé (1910).

Emery (1885) protests that Grosglik's conclusions would have been justified only if he had studied the forms upon which Emery's observations were based, or had his investigations extended over a wide range of bony fish. He admits that although his specimens of Zoarces were large, they may not have been "adult" in the strict sense of the term, but he reiterates with great positiveness that the observations on *Fierasfer* were made on adults.

The last word in this controversy is spoken by Grosglik (1886), who now accepts Emery's description of the pronephros as the chief excretory organ of adult *Fierasfer*. Grosglik considers this condition abnormal, and probably due to the quasi-parasitic life of the animal. (Emery, he says, conceded the influence of parasitism on other organs.)

Active functioning of a persistent pronephros is also claimed for several genera of the Macruridae described by Holt and Calderwood (1890-1891). In *Macrurus rupestris*, the kidney is divided in front of the bladder, the branches going into the head region "where they exhibit pronounced renal or pronephric characteristics. This head-kidney has every appearance of being perfectly functional." Uriniferous tubules are said to be numerous in any section of the organ. A large ureter(4) is described as passing dorsal to the air-bladder, and traceable anteriad to the head-kidney. This statement seems to be made from observations on gross morphology only, so that the question of a

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(4) The term "ureter" which is frequently used to apply to any excretory duct is objected to on the ground that only the duct of the metanephros, which has a distinctive mode of origin, should be so designated. The terms "pronephric" or "archinephric" duct will be used as referring to the primitive excretory duct of the pronephros. After mesonephric tubules have acquired a union with this structure, the posterior portion of the duct from the mesonephros to the cloacal region will be termed the "Wolffian duct". The anterior end remains the pronephric duct.

potentially functional connection between "ureter" and pronephros is in doubt. In M. larvis and M. caelorhynchus, however, the thickened masses which "probably represent persistent head-kidneys - - appear to be degenerate and functionless in the adult." Holt and Calderwood are inclined to correlate the (supposedly) functional state of the pronephros in M. rupestris with the fact that these forms, and others in which a persistent pronephros is described (Fierasfer, Dactylopterus), inhabit considerable depths. That a functional head-kidney is more common in deep-sea fishes than in shallow-water forms, is claimed by these authors, who point out that other modifications similar to this retention of a larval organ are known to have been produced by depth. It is stated further that while pelagic or shore fishes may also have a head-kidney (the forms described by Balfour are mentioned), in these cases it is degenerate and functionless. The comparative data on deep-sea and littoral forms, however, seems not sufficiently extensive to support the theory of Holt and Calderwood.

Calderwood (1891-1892) places Dactylopterus volitans among those forms possessing a functional pronephros in the adult. The swim bladder in this form is divided into two regions; the primary large and muscular; the secondary, which is thin-walled and situated above and anterior to the primary. The pronephros fills a pyramidal cavity of bone below and in front of the air-bladder. The pronephros is said to be entirely separated from the body kidney, but later the somewhat obscure statement is made that the "communication between the two is maintained by the canal formed in the ventral surfaces of the anchylosed first four vertebrae of the spinal column." No mention is made of a pronephric duct, yet Calderwood considers the pronephros "apparently a functional kidney", and it is difficult to conceive of a pronephros functioning without some such connection with the exterior.

The mesonephros of Dactylopterus is also functional, and shows a greater number of tubules than the pronephros. "This difference", Calderwood remarks, "may go to show that in Dactylopterus the degeneration of the pronephros is only commencing, but I think the conditions justify me in believing the organ to have a renal function."

In Cyclopterus lumpus, however, Calderwood finds degenerative changes occurring with the development of sexual maturity. These changes culminate in the formation of a mass of granular tissue, with empty spaces denoting the former position of tubules. It is thus inferred that complete degeneration and loss of function of the pronephros occur in some forms with the onset of sexual maturity, while in individuals of the same age of certain other species (e.g. Dactylopterus) there are few or no signs of degeneration, the pronephros remaining functional.

Guitel (1906) is not inclined to admit that the presence of tubules in the kidneys described by Calderwood and by Holt and Calderwood establishes the fact that these organs, even though functional, are persistent pronephroi. He maintains that to demonstrate beyond doubt that one is dealing with a pronephros, the presence of the single large pronephric glomerulus, and its continuity with the "ureter" must be established. He is therefore unwilling to consider the organs to be other than of a mesonephric character, in the absence of the positive proof which he demands.

Five genera of the family Gobiesocidae (Lepadogaster, Caularchus, Gobiesox, Syciases, Chorisochismus) are placed by Guitel (1906) among the forms in which the pronephros persists and functions in the adult. Of these he says, "Partout le pronéphros persiste jusque chez l'adult et reste parfaitement fonctionnel pendant toute la vie." The pronephros of these forms is described as containing a giant glomerulus supplied by a pronephric artery of varying length. The pronephric duct is in direct continuity with that of the mesonephros, and while the lumen of the former is subject to great variation in diameter, (8 to 200 microns), it is always open.

The development of the kidneys of the Gobiesocidae was not studied by Guitel, but he states that the essential characteristics of the embryonic pronephros as described by other workers are perfectly preserved in the adults of the nine species which he studied. These characteristics may be summarized as: a segmental duct in the form of a rectilinear tube terminating anteriad in a single nephrostome which is immediately preceded by a U-bend in the duct; the nephrostome opening to a pronephric chamber which represents an isolated part of the coelom and encloses a "Malpighian glomerulus."

That the pronephros persists in the adults of the forms in question and is "perfectly functional" is Guitel's conclusion.

On the basis of Guitel's criterion, "Fierasfer, Zoarces and the nine Gobiesocides studied here are actually the only Teleosts in which the persistence of the pronephros has been definitely demonstrated. However, a reservation must be made concerning Zoarces, because Emery is not certain that he was dealing with completely adult animals."

Audigé (1910) contends that the observations of both Guitel and Emery must have been made on immature specimens, for in spite of the wide range of material covered by his own investigations, he has never seen the type of kidney described by them in animals with well-developed genital organs. He concedes, however, the occurrence in the post-larval state of certain Teleosts of a functional pronephros.

The question of a functional pronephros in the adult Teleost is largely one of historical interest. It embraces two theories which modern investigation has shown to be unsupported by conclusive evidence:

1. The pronephros constitutes the only functional kidney in certain Teleosts. This was first stated by Hyrtl, and later supported by Calderwood.

2. The pronephros persists in the adults of some species, in conjunction with a mesonephros which may or may not be well-developed. Emery, Calderwood, Holt and Calderwood, and Guitel describe such a condition. Anatomists and embryologists (e.g. Kingsley, Graham Kerr)

commonly state that the pronephros functions throughout life in Fierasfer, Dactylopterus, and Lepadogaster, thus supporting Emery, Calderwood, and Guitel. Special workers in the field, however, question the evidence of these authors on the ground that in most Teleosts studied, degenerative changes in the pronephros occur late, and the work in question may have been based upon individuals which were only seemingly adult.

In view of these facts, the only statement compatible with the evidence is the admission that in only a few of the many Teleosts studied, the pronephros appears to be a functional renal organ.

Persistence of the pronephros as an organ of modified function

The adult "head-kidney" has already been described briefly as an organ situated anterior and dorsal to the air-bladder. Histologically, it consists of a spongy vascular tissue containing few or no renal tubules. Although it has generally been assumed that the head-kidney represents the persistence of the larval pronephros, conflicting opinions occur in the literature. These may be classified under three heads.

1. The structure described in the adult is the "homologue" of the larval organ, which has undergone structural transformation and modification of function.

2. The "head-kidney" is composed of diverse elements, differing in origin. One or more of its component parts may be derived from the pronephros, although the organ as a whole is not.

3. The so-called "head-kidney" does not correspond in structure, function or development to the pronephros. The proponents of this view imply that the "head-kidney" represents a structure of independent origin, but they do not describe its development.

Returning to a detailed consideration of these theories, it is proposed to review the existing evidence for the "homology" of the head-kidney and the pronephros, to describe the nature of the structural transformation, and to consider the various suggestions which have been made as to new functions acquired by the organ.

The embryological investigations with which the author is familiar have in general been restricted to a particular period in the ontogeny of the excretory system, and accordingly do not furnish descriptions of a complete series of stages which would indicate whether the embryonic pronephros corresponds to the adult organ termed the "head-kidney". Evidence of a non-embryological nature has been supplied by Audigé in his treatise on the kidneys of Teleosts. The proofs which he offers are of three types: ontogenetic, anatomical, and physiological.

The ontogenetic proofs result from a comparison of young forms with adults. The distinction between the two groups rests upon the first functioning of the genital organs. The pronephros consists of two types of tissues: (1) Glomerular tissues and tubules, and (2) An interstitial tissue (lymphoid tissue) enveloping the other elements of the organ. In passing from young to adult forms, a marked increase in lymphoid tissue is apparent, the glomerular and tubular elements being compressed and invaded by the former until their complete disappearance occurs. It is thus evident that the interstitial lymphoid tissue is an important constituent of the pronephros. Although our physiological knowledge is too limited to permit us to evaluate its functional importance, it cannot be considered merely an accessory. Its volume bears (roughly) an inverse ratio to that of tubules and glomerulus. However, the lymphoid tissue is not considered by Audigé a simple replacement tissue, because it is co-existent in the embryo with the other elements of the pronephros. Audigé concludes that the anterior lymphoid organ of the adult is "l'homologue du tissu lymphoïde pronéphrotique embryonnaire", because it corresponds in structure, relation to the general topography of the organism, and relation to the renal tissue.

The anatomical proofs of the "homology" between pronephros and the anterior lymphoid organ are concerned with the vascular relations of the anterior kidney. Comparison of young individuals with adults of the same species, and of a number of adults of different species, indicates that the arterial vessels supplying the anterior kidney of the adult represent the persistent pronephric vessels. The pronephric glomerular artery is formed in the embryo by the anterior and superior branches of the mesenteric artery. In the adult, the region of the anterior kidney, whether glomerulus and tubules are present or absent, is supplied by these same anterior branches. The "homology" of the blood vessels is interpreted as implying the "homology" of the regions or structures drained by them. - - "Le rein antérieur glomérulaire des jeunes, le rein lymphoïde supra-cardiaque des adultes, les pronéphros glomérulaires des Gobiesocidés de Guitel et des animaux d'Emery, sont tous homologues; ils résultent d'une ébauche commune, persistance du pronéphros embryonnaire."

The physiological evidence constitutes negative proof that the anterior kidney cannot be considered as a portion of the mesonephros. The method of circulation of the two renal organs, pronephros and mesonephros, is shown to be entirely different. The circulation of the anterior kidney is direct, i.e., blood brought into the kidney from the aorta by way of the pronephric arteries is carried away through the posterior cardinal veins. This circulatory cycle is true of the anterior kidney in any of its familiar modifications of form, whether glomerular, glomerular and lymphoid, or lymphoid alone. In the mesonephros, however, the greater part of the blood which passes through the organ is venous. The vessels of the mesonephros constitute a renal-portal system. The difference between the two systems may be indicated graphically:

PRONEPHROS:

Pronephric artery - pronephros - posterior cardinal vein.

MESONEPHROS:
Caudal vein
or
Intervertebral vein - mesonephros - posterior cardinal vein
or
Intercostal vein

It is true that the mesonephroi of some forms contain glomeruli supplied by arterial branches, but in every case these arteries are of relative unimportance, by far the greater part of the blood being carried by the veins.

From these proofs Audigé concludes without reservation that the "head-kidney" (rein antérieur) represents the persistence of the embryonic renal organ.

The work of other authors who subscribe to this theory has already been described (Calderwood, 1891; Holt and Calderwood, 1895). To these may be added Haller (1908) who distinguishes two types of pronephroi; the first, glomerular and tubular, in the young forms; the second, in adults, chiefly lymphoidal, but showing various degrees of degeneration of the tubules and of the development of lymphoid tissue.

According to Grosglik, the head-kidney is a composite of two structures, the reduced remnants of the embryonic pronephros intermingled with the cortical section of the supra-renal body. Grosglik regards the latter as derived from the lymphoid tissue which in the embryo surrounds the true renal elements, the tubules and glomerulus. Grosglik agrees with Emery (1882) that the lymphoid tissue develops from a cellular blastema arising from the peritoneal epithelium. This group of cells serves in part as a building material for the segmental tubules of the Wolffian body, but the part unused in this manner remains practically unchanged and constitutes the lymphoid tissue of the mesonephros. The latter may be traced also into the adult head-kidney where, according to Grosglik's somewhat fragmentary observations, it becomes the cortical portion of the supra-renals. This tissue, which is usually described as comprising most if not all of the organ known as the "head-kidney", is then regarded as "homologous" with the lymphoid tissue which arose with the renal elements, but not as "homologous" with the pronephros itself. This differs from Audigé's view in that the latter regards the lymphoid tissue as an integral part of the pronephros in the embryo. Grosglik concludes that only such remnants of pronephric tubules as may remain scattered through the lymphoidal substance of the head-kidney may be regarded as representing the pronephros.

Emery (1882) seems to support this view when he states that the head-kidney of the adult may or may not be "homologous" with the pronephros, the "homology" depending upon the presence of glomerulus and renal duct, as in Fierasfer and Zoarces. Since, in the cases under consideration, the absence of glomerulus and duct in a functional state is characteristic, Emery must be listed with those who believe there is no "homology" between the anterior lymphoid organ and the pronephros.

Weldon (1884) considers the reduced pronephros of adult

Bdellostoma to be represented by the supra-renal bodies, and supposes that a similar condition exists in the Teleosts. Grosglik (1886), however, makes a distinction between his interpretation of the "homology" of the supra-renals and that of Weldon. The latter, he says, considered the supra-renals to be derived from the glandular part of the pronephros, while he himself "homologizes" the supra-renals and the mesodermal mass of lymphoid tissue, the embryonic origin of which was described by Emery.

It is doubtful whether any such distinction was intended or understood by Weldon, for at the time of his communication little was known about the development of the organ, and there is nothing in his paper to indicate that he had any embryological basis for his theory. Weldon states that he hopes to demonstrate in the future an intimate relation between the Wolffian body and the supra-renal bodies, but admits such proof is now lacking. The organ which he describes in Bdellostoma corresponds in all essential particulars to the anterior lymphoid organ of Teleosts except that there appear to be a greater number of tubule remnants persisting, and there are also traces of a central duct. Lymphoid tissue is described as surrounding the posterior end of the central duct and forming a sort of capsule around the glomerulus. It may be inferred from this that the proportionate amount of lymphoid tissue in the kidney of Bdellostoma is less than that in Teleosts, but there is no statement in Weldon's paper to indicate that he did not have the whole organ in mind when he suggested the "homology" with the supra-renals.

McKenzie (1884) opposes Weldon's theory that the anterior kidney represents the supra-renal bodies, since he finds the supra-renals in <u>Ameiurus</u> as a single pair of bodies on the lateral surface of each kidney (mesonephros?), but which, he states, appear to have no connection with it.

Emery (1885) concedes that the lymphoid tissue of the Teleost kidney may contain the same elements as the supra-renal cortex of Amniotes, but in an undifferentiated state. He denies that a "true homology" may exist.

Vincent (1897) opposes the "homology" of the supra-renals and the anterior kidney. He summarizes his views of the relation of the supra-renals to the head-kidney in Teleosts and Ganoids as follows: Most Teleosts have a "lymphatic head-kidney", a lymphoid organ with a probable blood-destroying function. The kidney of fishes is composed of two kinds of tissue, secreting tubules and adenoid intertubular material. The head-kidney of Teleosts is not a specialized portion of the primitive kidney, but an accumulation at the anterior end of the organ of the lymphoid tissue. No anatomical or physiological relationship of any kind exists between the supra-renals in Teleosts and the lymphatic head-kidney.

Guitel (1906) describes the occurrence of definite supra-renal capsules in <u>Lepadogaster</u>. He considers them, however, as entirely distinct from the anterior kidney, and the two are obviously co-existent in this case.

Balfour (1881) (1882) was one of the first to point out the lack of renal elements in what was then supposed to be a functional part of the excretory apparatus, the head-kidney. He studied sections of the kidneys of Acipenser (the sturgeon), Esox lucius (the pike), Osmerus eperlans (the smelt), Anguilla anguilla (the eel), and Lophius piscatorius. He found that the kidney was in general divided into a posterior region containing uriniferous tubules and Malpighian bodies, and an anterior- (head-) kidney composed of lymphoid tissue with few or no tubules. The few tubules which he found in the smelt he regarded as the "functionless remnants" of the pronephros. (Balfour's interpretation of his findings in regard to Lophius has already been mentioned.) He takes the position that in representative Teleosts at least, no functionally active remains of the pronephros persist in the adult, the organ being confined to the larval state. He declines to consider the anterior lymphoidal swelling as the persistence of the pronephros. He does not, however, attempt to identify the lymphoid tissue with any embryonic structure, and his evident lack of knowledge on the development of the tissue did not permit him to appreciate the fact of the close relation which exists between lymphoid elements and renal tubules even in the embryo. He is rather inclined to regard it as an entirely separate element, which cannot be "the absorbed remnant of the pronephros" because of its abundance, its vascular character, and its presence before the atrophy of the pronephros. The essence of Balfour's conclusion (1882-b) is that the pronephros must now be regarded as a purely larval organ in all the Vertebrates, since Teleosts and Ganoids were the only groups in which it was previously supposed to persist.

Parker (1882) in a brief note concurs with the opinion of Balfour that no functionally active remains of the pronephros occur in Teleosts, and he further comments that in some the supposed "head-kidney" has the same structure as the mesonephros. This he attributes to the forward growth of the latter above the air-bladder to take the place of the pronephros.

As has already been pointed out, both Emery and Guitel deny the "homology" of the anterior lymphoid organ with the pronephros inasmuch as it does not contain a functional glomerulus and duct.

Whatever view is taken of the significance of the head-kidney, the fact remains that a structural transformation has occurred, by means of which an organ of predominantly lymphoid character has come to occupy the position formerly held by the embryonic pronephros.

Theories of the origin and function of the lymphoid tissue

The origin and development of the lymphoid tissue is subject to a diversity of interpretations. Semper (1875) is said by Audigé to have attributed the presence of lymphoid tissue in the pronephros and mesonephros to the degeneration of the capsules of the Malpighian glomeruli. Emery (1882), however, claims an epithelial origin for the tissue. In embryos of Belone and Zoarces he recognized between the aorta and the cardinal vein a mass of cells in contact with the renal tubules. This he calls the "blasteme cellulaire du rein." The embryos were too advanced to give any clue to the origin of the blastema, but in young specimens of Zoarces, he found it to be in continuity with the peritoneal epithelium. The blastema was traced into the head-kidney to the glomus of the pronephros, where it was continuous with the tissue of this structure and with the lining of the capsule itself. It is believed by Emery to give rise to the renal tubules of the mesonephros. Not all of the blastema, however, is thus utilized. The unused portion is thought to constitute the lymphoid tissue of the adult kidney, in other words, the portion which remains unaltered in the midst of the completely formed tubules, and which is traversed by large blood spaces with thin walls. These relations are clearly shown in embryos of Zoarces, and correspond to those of the lymphoid tissue of the adult.

Felix (1906), suggests that the lymphoidal tissue (Pseudolymphoides Gewebe) arises from the walls of the interrenal veins. A thickening of the walls of the blood vessels occurs by the proliferation of a solid cell-mass which comes almost completely to fill the spaces between the coils of the pronephric duct. By its excessive growth, it unites the two pronephroi of the opposite sides into an unpaired organ.

A view not altogether compatible with that of the embryologists who point to the early development of the lymphoid tissue is presented by Verne (1922). In his opinion, the development of this element is correlated with the retardation of the circulation of the blood which must result from the peculiar structure of the kidney. The reticular lymphoidal tissue appears simultaneously with the development of the rich afferent venous system of the mesonephros. The inference is that at this time the pronephros has already undergone considerable degeneration, although Verne is not clear on this point. The mesonephros, however, is described as filled with a network of venous capillaries which form intermediaries between the afferent veins and the cardinal veins. The capillaries traverse a reticular tissue "in every way comparable to the spleen of Mammals", and expand into true sinuses. The blood must pass slowly through a structure of this nature. This retardation results, according to Verne, "as in many other organs, in the accumulation of free elements and the development of the tissue called in the fish, pseudolymphoid." The destruction of non-functional remnants of the pronephric tubules in the cephalic region is thought to be effected by the invasion of numerous phagocytes of lymphoidal tissue from the mesonephric region. Verne emphasized the fact, however, that such invasion of tubules by lymphatic elements does not take place until the tubules have ceased to function and have become degenerate. Penetration of lymphocytes into the functional tissue of the adult kidney (Verne evidently means into the walls of the tubules themselves) was never observed.

This theory obviously fails to account for the presence of a great abundance of lymphoid tissue in the embryonic pronephros.

Verne points out an interesting correlation (first established by Audigé) between an abundance of lymphoid tissue and a scarcity of Malpighian glomeruli. This relation may be expressed in the statement that the development of lymphoid tissue is inversely proportional to the number of glomeruli. For example, in a typical mesonephros of predominantly venous circulation, with few or no Malpighian bodies, the lymphoidal tissue might be said to form the matrix in which are imbedded the renal tubules and the blood vessels. In those Teleosts in which a posterior kidney (thought by many to represent a true metanephros) is developed, the glomeruli are numerous and the lymphoid tissue is reduced to such an extent that the tubules are practically contiguous. Verne's mechanistic explanation of the development of the lymphoid tissue might also explain how the tissue diminishes as the glomeruli make their appearance. As arterial connections are developed the circulation of the blood is transformed from a sluggish to an active state, and the conditions favorable to the production of lymphoidal elements no longer exist.

Audigé ascribed to the lymphoid tissue an important role in the destruction of worn-out renal cells. It was his view that where the glomeruli were lacking, or few in number, the urinary flow would not be sufficient to carry away these cells. The recent work of Marshall and Smith (1930) provides important evidence that the glomeruli of Teleosts, as those of higher vertebrates, are important in the elimination of liquid waste. The development of glomeruli and the resulting increase in the excretion of liquid waste matter would render such a service on the part of the lymphoid tissue no longer necessary. "L'inutilité du tissu lymphoïde entraîne sa disparition."

Verne and Audigé agree that the lymphoid tissue constitutes a true organ. According to Audigé, however, its function is purely renal, serving the physiological purpose of the Malpighian glomeruli of more highly developed kidneys.

Drzewina (1905) established the functional correlations in the Ichthyopsida between the spleen and the localizations of lymphoid tissue characteristic of that group. Verne further points out the resemblance in structure and type of circulation between the lymphoid organ and the spleen. He considers Audigé's hypothesis of the action of the lymphocytes on the cells of the renal tubules problematic. He states that he has never observed in Lophobranchs such a functional relation.

Policard and Mawas (1907) suggested that the red corpuscles of the blood are probably destroyed in the lymphoidal tissue of the kidney. Audigé supports this view, and Verne apparently concurs in the opinion that the lymphoid tissue is the seat of erythrolysis, a function in mammals performed by the spleen.

Degeneration of the pronephros

No careful study of the degenerative changes in the pronephros fails to emphasize the fact that the decrease in number of tubules is accompanied by a marked increase in the volume of the lymphoid tissue. Felix (1906) describes the degenerative changes of the pronephros of the trout and salmon. These changes are initiated as soon as the mesonephros becomes fully functional, which in these forms occurs between the 195th and 192nd days after hatching. On the 192nd day the arterial supply of the pronephric glomerulus is cut off and this organ subsequently degenerates. A week later both nephrostomes are greatly reduced in size. The pronephric duct soon shows a reduction in diameter, and here and there its lumen is obliterated. The pronephros remains in this condition until about 617 days after fertilization, at which time the pronephric chamber and the pronephric tubules disappear. The pronephric duct, however, persists. The two pronephroi which in the embryo have been united into an unpaired organ by the pseudo-lymphoidal tissue, now separate, this split continuing throughout the extent of the head-kidney to the mesonephros. During the course of such degeneration the mass of pseudolymphoidal tissue is increased, so that no diminution in the volume of the head-kidney results.

Most authors state simply that the degeneration of the pronephric tubules occurs at the onset of sexual maturity. An account somewhat less precise than that of Felix was given by Grosglik (1885).

He states that the first indication of deterioration occurs soon after the formation of the mesonephros and the fuller development of the other organs. These changes appear first in the region of the cardinal vein, the adjacent tubules losing their lumina. The walls of the tubules meet, their cylindrical cells round up and form a heavy border around the venous walls. These changes extent into the posterior region of the head-kidney, where the cells of the tubules become small and their boundaries are broken down. The anterior end of the kidney (adjacent to the glomerulus) shows no such deterioration, but remains unchanged until the time of sexual maturity. Its tubules, however, must be non-functional, since their continuity with the Wolffian duct is lost. The disappearance of tubules and glomerulus in this region occurs late, and their absence only in completely adult individuals.

The embryological development of the Teleostean pronephros

The investigation by Swaen and Brachet (1900) of the pronephros and other organs derived from the mesoderm repays careful consideration. A description of the conditions found in the trout was first published by these authors. A later investigation on several other Teleosts showed the mode of formation of the pronephros in the trout to be a-typical in several important respects, and a modified interpretation of wider application to Teleosts in general is given by Swaen and Brachet in 1902.

Because the description of the development of the trout is given in minute detail, however, it forms a necessary background for the understanding of the modifications which occur in other forms.

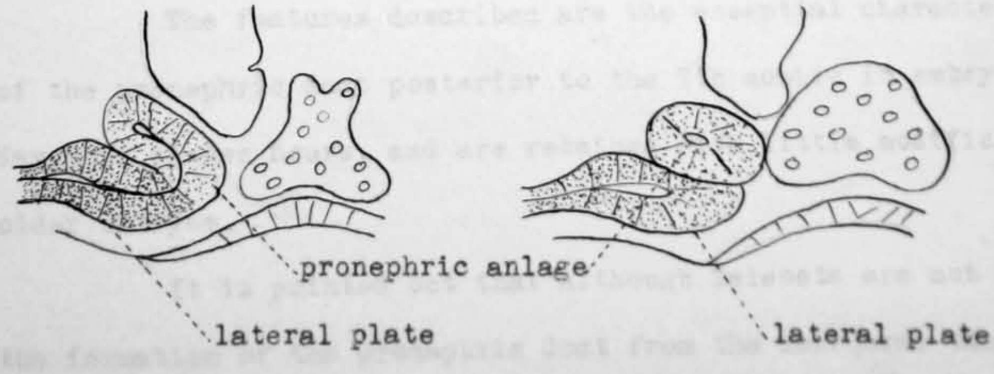
The whole pronephric anlage in the trout is said to be formed by the simple isolation of the internal part of the splanchnocoele from the fourth somite to the cloaca.

The mesoderm in the youngest embryo studied (possessing four somites and the beginning of the fifth) is interpreted as consisting of a part designated as "interne somitale" (the somite-producing portion, or epimere), and the primitive lateral plate, situated externally. The internal region subsequently gives rise to the myotomes and the sclerotomes. The primitive lateral plate undergoes division into the secondary lateral plate (apparently the region usually designated by other authors as the hypomere, and the intermediate mass, or mesomere. This latter constitutes the tissue in which the blood vascular system later forms. The secondary lateral plate is differentiated into an internal part, the pronephric anlage, ("le canal excréteur"), and an external, the lateral plate. The secondary lateral plate is evidently the entire hypomere. In the region of the pronephric anlage, the inner (medial) angle of the coelom and the somatic and splanchnic mesoderm which form its walls are more or less completely isolated as the pronephros and its duct. The "lateral plate" represents only the external portion of the primitive hypomere. In the posterior end of the embryo, and in the region anterior to the pronephros, no differentiation of the "secondary lateral plate" occurs.

The region which is separated as the excretory duct is thus primitively a part of the splanchnocoele, and since it is formed in the inner angle of the latter, it is considered by Swaen and Brachet that both somatic and splanchnic epithelium participate in its formation. The belief of many authors that the duct is formed only of somatic mesoderm is attributed by Swaen and Brachet to the fact that the inner angle of the splanchnocoele undergoes a rotation (which will be described later), by which the pronephric anlage comes to lie above and rest upon the somatic mesoderm, and thus might give the impression of being derived from it.

The term "canal excréteur" seems to be applied to the entire pronephric anlage as it first appears. The posterior part of the "canal excréteur" from the 7th somite to the cloaca, represents the medial part of the hypomere which has become isolated from the main portion of the coelom and its walls. The anterior pronephric region, (from the 7th to the 4th somite), which undergoes a similar, though more gradual isolation, is designated "the pronephric chamber" to distinguish it from the posterior. In their development, however, all parts of the pronephric anlage or "canal excréteur" are similar,, so that the terms applied to anterior and posterior regions are for convenience only, and do not imply a different mode of origin. Anterior and posterior part alike represent an isolated part of the coelom, -"une chambre pronéphrétique rudimentaire". By a course of development which will be described, the anterior part (from the 4th to the 6th somite, inclusive,) becomes modified into a true pronephric chamber ("la chambre pronéphrétique vraie"), while the posterior part remains rudimentary. After this time, Swaen and Brachet seem to apply the term "canal excréteur", or "chambre pronéphrétique rudimentaire", to the region extending posteriad from the 7th somite.

The development of the posterior end of the pronephric duct of the trout differs little from the condition found in other Teleosts. The internal portion of the hypomere becomes isolated from the somatic and splanchnic mesoderm which bounds the coelom by an oblique plane of cleavage. This process of separation, extending from anterior to posterior as the embryo advances in development, results in the formation of a long cellular cord, or rod, lying between the definitive lateral plate and the intermediate mesoderm, and representing the internal extremity of the hypomere.



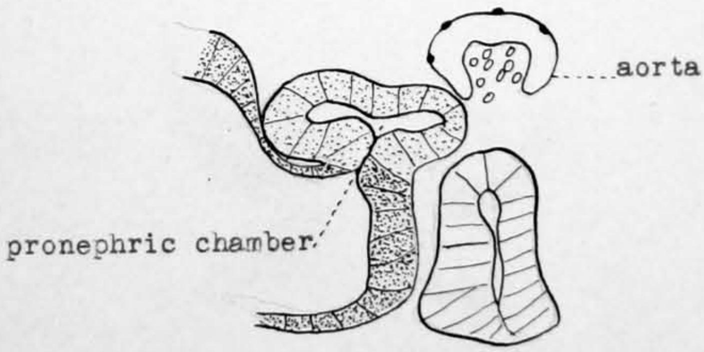
The duct grows posteriad at the expense of both somatic and splanchnic layers. The lateral plate soon slips under the duct and raises it, so that the superior border of the duct forms a projection between the intermediate mass and the internal border of the lateral plate. Under these conditions, its narrow ventral surface may be distinguished with difficulty along the inferior surface of the lateral plate. Upon superficial examination it might be concluded that the canal was formed at the expense of the upper layers of the lateral plate, in other words, wholly from somatic mesoderm. Careful observation shows that the cells of the tapering inferior border of the duct extend to the inferior (splanchnic) surface of the lateral plate.

The cells constituting the duct now arrange themselves about a central lumen, and the whole structure, except in the anterior region, modifies its primitive oval shape and becomes circular in cross-section. The caudal end of the duct, becoming separated from its place of origin, approaches the cloaca, into which it soon opens. (Brachet, 1921).

The features described are the essential characteristics of the pronephric duct posterior to the 7th somite in embryos of ten days and eleven hours, and are retained with little modification in older embryos.

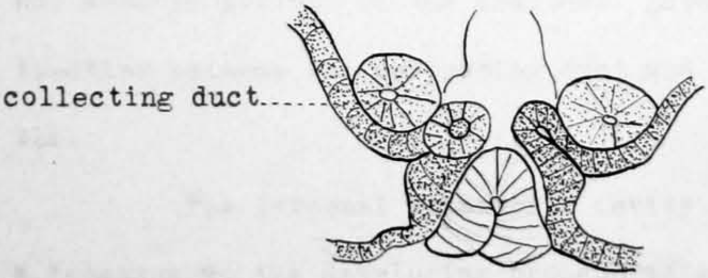
It is pointed out that although Teleosts are not alone in the formation of the pronephric duct from the mesoderm, they are the only Vertebrates in which the splanchnic layer of mesoderm intervenes in its formation.

In the anterior part of the pronephric anlage, changes of great significance are initiated. Isolation from the splanchnocoele is accomplished very gradually, the cavity of the pronephric chamber remaining for a long time in continuity with the splanchnocoele proper. In the region of somites 4, 5 and 6, the anlage increases in size through the growth of its somatic and splanchnic walls. Changes in position caused by the growth of the lateral plate toward the median line result in the rotation of the anterior end of the pronephric anlage. The somatic wall is carried outward; the splanchnic, inward. The cavity, formerly oblique, becomes horizontal; the former internal and external walls become superior and inferior respectively. The "true pronephric chamber" is thus formed.



These changes of configuration are produced in the region of the 5th and 6th somites; at the level of the 4th, the pronephric chamber terminates in a cul-de-sac. During the rotation of the chamber, communication between its cavity and that of the splanchnocoele is maintained by a vertical slit between somatic and splanchnic walls.

The chamber now becomes separated more or less sharply into an internal splanchnic and external somatic part. During further development this division is accentuated, and is more marked in the posterior half of the chamber. Isolation of the external and internal portions is effected by an antero-posterior constriction. The external cavity is continuous with the pronephric duct. The internal loses all trace of a lumen and becomes a circular mass of cells in contact with the somatic and splanchnic walls of the lateral plate. A slit-like space still separates the coelomic walls, but does not penetrate the chamber wall. All communication with the splanchnocoele is thus lost.



The separation of the internal from the external part of the chamber proceeds from its posterior wall anteriad, and halts in the median region of the chamber. At this level, and cranial to it, however, there may still be distinguished an elongated external half which is continuous with the pronephric duct, and an internal half into which the glomus subsequently comes to project.

The external part of the chamber is bounded chiefly by somatic epithelium, the internal by splanchnic.

To the somatic external part of the chamber which is in communication posteriorly with the pronephric duct, Swaen and Brachet attribute the value of a collecting duct ("canal collecteur", -"Sammelrohr"). In the median region of the pronephric chamber (the level of the 5th somite), the external somatic portion becomes incompletely separated from the internal chamber, and constitutes a segmental tubule (un canalicule segmentaire) which communicates posteriorly with the collecting duct. Segmental tubule and collecting duct, therefore, compose the anterior and posterior region, respectively, of the external somatic portion of the chamber. Later development renders a distinction between the collecting duct and the pronephric duct impossible.

The internal splanchnic cavity of the chamber now acquires a relation to the developing pronephric glomus. This structure pushes before it the inferior wall of the chamber, and comes to fill the invagination thus formed.

The formation of the pronephros is now complete. It consists of a single segmental tubule in continuity on one hand with a collecting duct, and opening, on the other, into a closed pronephric chamber, into the interior of which projects the glomus. The collecting duct is continuous posteriorly with a rudimentary pronephric chamber which performs the function of an excretory duct, and is so designated.

"The first anlage of a renal organ in the Teleosts is nothing but a long pronephric chamber extending from the level of the 4th somite to the cloaca. Throughout the greater part of its length, it undergoes no important modification, and functions as an excretory duct. Only in its anterior or cranial part, it envelops a vascular glomerulus on one hand, and on the other constitutes a segmental tubule and a short collecting duct (Sammelrohr); it becomes a true pronephros."

These investigations on the trout were later extended by Swaen and Brachet (1902) to a number of other Teleosts. From their mode of development, these are divided into two groups.

The members of the first, including embryos of Leuciscus cephalus, and of Exocoetus volitans, are in general similar in their development to the embryos of the trout.

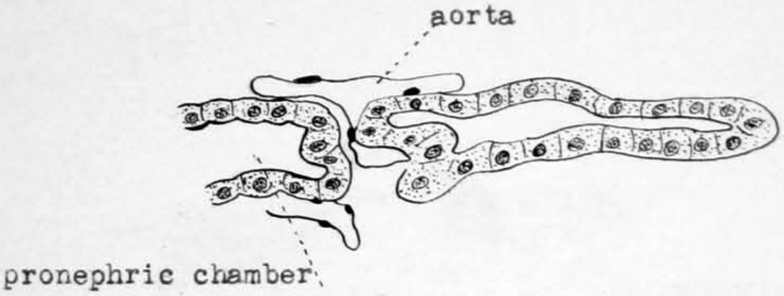
The second group, in which are Clupea sprattus, Rhombus, Solea vulgaris, Pleuronectes microcephalus, Trachinus vipera, Caranx trachurus, and Callionymus lyra, is distinguished by the much lower degree of development exhibited by the intermediate masses, and by the fact that the red blood corpuscles are undeveloped at the time of hatching. All the embryos in this group show great similarity in their mode of development.

The eggs of all these species are small, and the embryos of both groups develop very rapidly, hatching from three to five days after fertilization. The formation of tissues and organs thus takes place much more rapidly than in the trout, and a marked shortening in the processes of differentiation of the mesoderm results.

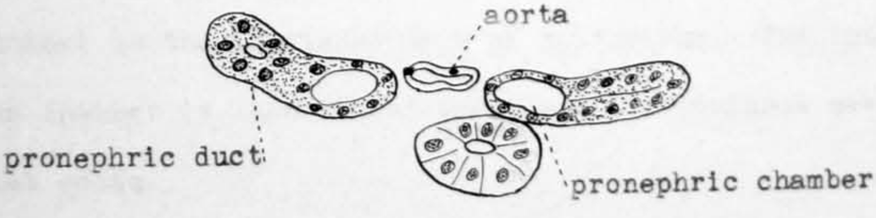
The influence of this speeding up of the processes of development upon the formation of the pronephros is especially apparent in the anterior, or pronephric chamber region. Here the anterior end of the pronephric anlage, which, from the moment of its isolation from the splanchnocoele, has been slightly more elongated at right angles to its long axis than the remainder of the excretory duct, continues to develop in this direction so as to extend toward the median line. It thus forms a transverse epithelial tube directed toward the aorta, adjacent to which lies its internal extremity. The external end of the tube is continuous with the pronephric duct. The transverse tube thus runs at right angles to the posterior, longitudinal portion of the duct.

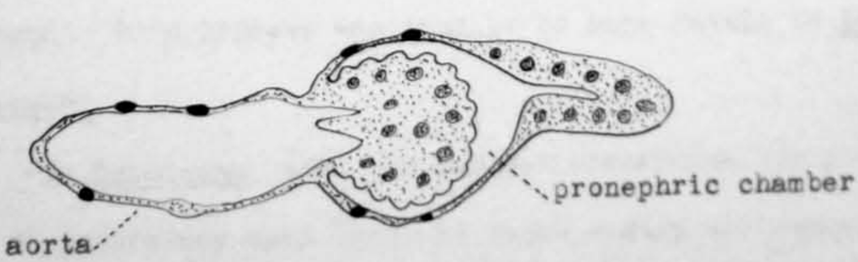
The configuration of the rounded internal end of the cavity varies with the species.

In Leuciscus it comes to lie in juxtaposition to that of the opposite side in the median line just beneath the aorta.

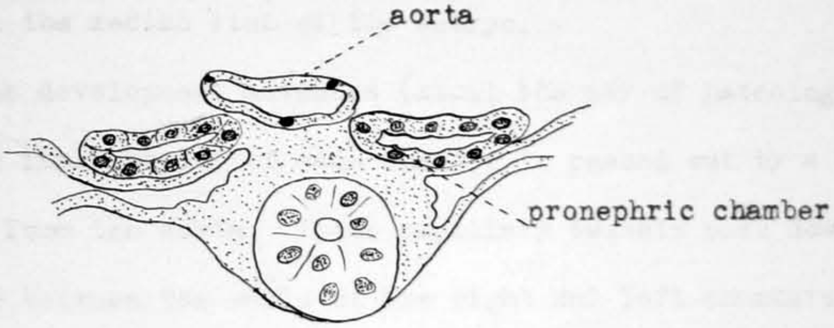


In Exocoetus and Solea, it lies in the same plane as the aorta and adjoining the external surface of its wall.





In Rhombus and Caranx, it ends at the inferior surface of the lateral border of the aorta.



The epithelial tube is composed of a wall of cubical cells which surround a more or less extensive cavity.

This space soon enlarges, the dilation of its internal half being especially marked. The lining cells undergo a change from the cubical to the flattened type of epithelium. The internal part of the chamber is thus transformed into a voluminous cavity, lined with flat cells.

A little later, the internal wall of the cavity is invaginated by the growth of the glomus, and a "corpuscle de Malpighi" is thus formed. This process was studied in some detail in <u>Leuciscus</u> and <u>Exocoetus</u>.

In <u>Leuciscus</u>, when the chamber containing the glomus is formed, the excretory duct bends at right angles and communicates with the chamber through a tubule. The diameter of the latter is short at its distal end (adjacent to the duct), but as it approaches the chamber the lumen of the tubule dilates, and the epithelial cells of its walls become flattened. The chamber is of ovoid form, and the heavy internal wall of the right chamber is in contact with that of the left in the median line of the embryo.

As development advances (about the day of hatching), the wall of the inner border of each chamber is pushed out by a vascular strand from the aorta. These capillary vessels push down in the median line between the walls of the right and left chambers, and in dilating produce an invagination of the inner wall of each chamber into its cavity. "This is evidently the beginning of the formation of a Malpighian corpuscle."

In Exocoetus the details of the process differ from that described for Leuciscus.

Here the transverse tubule, with its narrow lumen circumscribed by cubical cells, remains for a considerable time with its rounded internal border against the wall of the aorta. When a chamber is formed, its internal wall is still composed of thick cuboidal epithelium, although the cells of the remainder of the chamber become flattened. The cuboidal epithelium in contact with the aorta gives rise by proliferation to a spherical epithelial mass which extends into the cavity of the chamber. Meanwhile, the distal part of the chamber elongates into a tubule. Only in embryos of a considerably advanced stage of development does the aorta give rise to a capillary network which penetrates the epithelial structure described.

The entire pronephric anlage of all forms studied, including the trout, has the same essential composition, inasmuch as it is formed from the medial part of the hypomere, and represents an isolated portion of the coelom.

The marked differences in development between the trout and all other forms studied are localized in the extreme anterior end of the pronephros. They may be recapitulated as follows:

(a) In the embryos of all species studied, with the exception of the trout, the anterior end of the pronephric anlage (of short antero-posterior extent) develops at right angles to the pronephric duct toward the median line. A transverse tubule is thus formed, the medial portion of which expands into the chamber of the "Malpighian body." The latter is composed of the splanchnic elements which form the inner wall of the chamber. The tubule is lined by the somatic walls of the external half of the chamber, and is continuous posteriorly with the pronephric duct. The tubule is thus the homologue of the segmental tubule of other Vertebrates. There is no trace of a collecting duct in these embryos.

(b) In the trout, the length of the pronephric chamber corresponds to three somites. The anterior end terminates blindly. The median and posterior regions are differentiated into an external portion, the collecting duct, in continuity with the pronephric duct; and an internal, which forms the cavity of the "Malpighian corpuscle."

The segmental tubule establishes communication between the chamber containing the glomus and the collecting duct.

The greater antero-posterior extent of the pronephric chamber in the trout is considered by Swaen and Brachet an important factor in producing these differences in the development of the anterior end of the pronephric anlage.

Felix (1897)(1906), presents an account of the formation of the pronephric anlage of the trout and salmon, which differs from that of Swaen and Brachet chiefly in his interpretation of similar structures.

The pronephric anlage of an embryo of eleven somites consists of five solid outgrowths of the lateral plates, segmentally arranged, in somites three to seven. This primitive condition is of brief duration.

Immediately after the formation of the twelfth somite, the five "Zapfen" fuse into a continuous fold, in the formation of which both somatic and splanchnic layers participate, and thus the primary pronephric fold (primäre Vornierenfalte) is formed. The slit-like cavity of the fold communicates throughout its length with the coelomic cavity of the lateral plate.

From the 8th to the 10th somite, the anlage of the caudal end of the pronephric duct (primären Harnleiters) appears. Felix distinguishes two regions of the duct: the anterior end into which the tubules open, called by him the "Sammelgang", and the posterior, the "Endabschnitt des primären Harnleiters." The latter arises by the separation of primary lateral plate into three longitudinal strands, the lateral plate, the caudal end of the pronephric duct, and the "Venenstrang." These divisions appear to correspond respectively to the lateral plate, "canal excréteur", and "masse intermédiaire" of Swaen and Brachet. With the extension of this three-fold division of the primitive lateral plate, caudad, the pronephric duct is carried back toward the cloaca, into which it comes to open.

With the formation of the pronephric fold and the caudal end of the pronephric duct, Felix considers the pronephric anlage completed. Further developments are of a secondary nature.

The pronephric fold now becomes separated into dorsal and ventral portions by a fold which extends from the dorso-lateral to the ventro-medial wall. From the dorsal section of the primary pronephric fold arises the cranial end of the pronephric duct, -which is homologous with the collecting duct (Sammelrohr) of higher Vertebrates. The ventral section becomes the pronephric chamber.

In further development, the relative position of these parts is altered, so that the collecting duct becomes lateral, the chamber medial. At the same time, these two regions of the pronephric fold become virtually separated from each other, communication being maintained only in one place by the pronephric tubule (called by Felix the "Pseudovornierenkanalchen", because he does not consider it a true pronephric tubule.) From this reasoning follows his designation of the communication between the tubule and the chamber as "Pseudonephrostom".

The anlage of the "glomerulus" is next formed. According to Felix, the glomerulus does not project into the chamber, but is surrounded by the latter through the building of dorsal and ventral "horns". Each chamber (one on the right, the other on the left) sends out two diverticula toward the median line, one dorsally above the glomerulus, the other ventrally below it. The walls of the right and left dorsal horns meet in the median line above the glomerulus; the ventral horns unite in a similar manner below it. The blood vessels from the aorta, which constitute the vascular tissue of the glomerulus, unite posteriorly to form the mesenteric artery.

In brief recapitulation, it is the view of Felix that the pronephric fold arises by the fusion of a series of pronephric tubules. The tubules are formed by an outgrowth of the wall of the coelom, and the space enclosed by the pronephric fold must therefore be regarded as an evaginated or secondary body cavity, the chamber of which is newly formed and does not represent a part of the pre-existing coelom. The pronephric fold gives rise to the cranial section of the pronephric duct (Sammelgang), and the pronephric chamber. The orifice through which the chamber communicates with the main coelom is interpreted by Felix as the fused nephrostomes of all the pronephric tubules which originally made up the fold. He considers the pronephric chamber of the Salmon as of the internal type, representing, not an isolated part of the pre-existing coelom, but a diverticulum from it.

It is not proposed at this time to review the somewhat extensive literature dealing with the homology between the pronephric chambers of Teleosts and Amphibia, but it may be pointed out briefly that Felix considers the chambers of the two as "Ontogenetisch zwei vollstandig verschiedene Gebilde", which cannot be homologized. He classifies pronephric chambers as internal (the Salmon) and external (Amphibia). In this he declares he is in agreement with the earlier views of Hoffman and Rosenberg.

Apart from their disagreement on the manner of formation of the pronephric chamber, there seems to be no essential conflict between the account by Swaen and Brachet and that of Felix. The French embryologists attribute the theory of Felix to an error in observation on his part. The five pairs of "Zapfen" which he considers rudimentary pronephric tubules which are later to undergo fusion into the pronephric fold, they consider to be nothing but the intersegmental projection of the intermediate masses. He may have mistaken these for the prolongation of the lateral plates. A reconstruction made by Brachet and Swaen of the lateral plates and intermediate masses resembles that figured by Félix as the segmentally arranged "Zapfen" and convinces them of his error of interpretation.

According to Felix, the "Zapfen" fuse at the level of somites 5, 6, 7 and 8 to form the pronephric fold. Swaen and Brachet explain this apparent fusion by stating that in this region the intermediate tissues are less compressed by the somites and rest in contact with each other.

Apart from this major difference, the interpretations of Swaen and Brachet and of Felix are wholly reconcilable, differing only in the terminology applied to the same structures. The following terms, as used by these authors, appear to be equivalent:

Felix	Swaen and Brachet
primäre Vornierenfalte	chambre pronéphrétique
kaudaler Abschnitt des primaren Harnleiters	(canal excréteur (in restricted sense) ((posterior end of pronephric anlage.) (chambre pronéphrétique rudimentaire
kranialer Abschnitt das primaren Harnleiters	canal collecteur
Pseudovornieren kanalchen	canalicule segmentaire
Vornierenkammer	chambre pronéphrétique vraie
Pseudonephrostom	nephrostom

Burlend (1931) states that the pronephros and its duct arise in the Teleostei from a thickening of the "splanchnocoelic mesoderm". "There can be no doubt about this", he says, "because the thickening is not segmented and acquires at an early stage a continuous slit-like lumen which is open to the splanchnocoele. The thickening is therefore not of nephrotomic origin, and is evidently the anlage of the primitive nephric groove." Precisely what Burlend means by "splanchnocoelic" mesoderm is not clear, but the supposition that he means somatic mesoderm seems to be supported by his further description. The thickening is said to be converted into a groove in somites 5 to 10. Behind this region, it separates from the splanchnocoele mesoderm as a solid rod, which later acquires a lumen, and gives rise to the greater part of the "archinephric duct", i.e., the "Endabschnitt" portion. The groove remains open at the level of scleromyotomes 5 and 6 as the nephrostome of the solitary pronephric tubule. (Burlend differs from Felix here in regarding the connecting structure between the coelom and the anterior end of the duct as a true pronephric tubule. Felix designates this a "Pseudovornierenkanalchen". The "peritoneal funnel" of Felix thus corresponds to the "nephrostome" of Burlend.) The remainder of the groove from somites 6 to 10 separates from the splanchnocoelic mesoderm to form the Sammelgang portion of the archinephric duct. (The "collecting duct" of Swaen and Brachet in the trout was regarded as situated at the level of the 5th somite. What Burlend considers the "Sammelgang" they interpret as the anterior end of the pronephric duct.)

Burlend regards the archinephric duct in Anamniota in general as "differentiated from the somatopleuric mesoderm lining the primitive splanchnocoele." Swaen and Brachet state that the Teleosts are the only vertebrates in which the splanchnic layer intervenes in the formation of the pronephric anlage.

The development of the mesonephros

The development of the mesonephric tubules in their relation to the pronephric duct will now be considered briefly.

The mesonephros develops during the late embryonic or early larval life of most Teleosts. Its exact manner of origin is not definitely established.

Felix (1897)(1906 states that the first anlagen of mesonephric tubules occur on the 70th day after fertilization; the last on the 150th day. At this time general development has progressed to the point where all organs are laid down and functional, and the external body form is definitely established.

The tubules of the mesonephros are formed in three stages, and are designated as tubules of the first, second and third order.

In cross section, there is marked off from the notochord and the myotomes, a trapezoidal region in which are located aorta, cardinal veins, and pronephric ducts. The latter lie directly dorsal to the wall of the coelom, and are composed of cuboidal epithelium.

The tubules of the first order appear as solid masses of small cells, disposed in concentric layers. These buds appear first in relation to the median third of the pronephric duct, and extend gradually to its caudal third, but never cephalad. In the median third the buds are disposed metamerically, but caudad this regularity of arrangement is lost.

Two months after the hatching of the embryo the primary tubules are said to acquire a lumen which immediately breaks through into the duct. The tubules are formed in complete independence of the pronephric duct, and develop a relation to it only secondarily.

The blind end of each tubule expands into a sort of chamber in which a glomerulus develops. The vessels of the glomerulus are thought to arise from the intercostal arteries.

The origin of the cellular masses which constitute the anlagen of the primary tubules is in doubt, but Felix suggests they may have been derived from the coelomic epithelium.

It will be recalled that the "blastème cellulaire du rein" of Emery (1882) is also considered to have an epithelial origin. Emery derives both mesonephric tubules and lymphoid tissue from this blastema. Small groups of cells proliferate into a solid mass in contact at its ventral extremity with the renal duct and by its dorsal surface with the undifferentiated part of the blastema. These cylindrical masses acquire a lumen, become convoluted and develop a relationship to the circulatory system through the formation of glomeruli.

Sedgwick (1881) had previously stated that the mesonephric tubules of the Teleost are derived from mesodermal cells which adjoin the peritoneal epithelium.

While tubules of the first order are still being formed in relation to the caudal end of the duct, Felix states that secondary tubules arise in the cranial end. The anlagen of the tubules of the second order resemble those of the first in general form, arrangement, and manner of development. The exact origin of their cells is likewise in doubt. While the primary tubules develop only in relation to the dorsal surface of the duct, the secondary may arise near any point of its periphery. The primary tubules are restricted to the caudal half of the duct; the secondary may appear at any point along its length. Felix asserts that there is no possibility that primary tubules may give rise to secondary by proliferation or division. The secondary tubules soon break through into the pronephric duct.

The tertiary tubules resemble primary and secondary in histological structure and development. They are unique in the time of their appearance, and especially in the fact that they may arise in relation not only to the periphery of the duct, but to the walls of primary and secondary tubules as well. The lumen of a tertiary tubule may be in continuity with that of the pronephric duct, or of a primary or secondary tubule. Each develops a glomerulus. The increase in bulk of the mesonephros is chiefly due to the formation of tertiary tubules.

When fully developed, primary, secondary and tertiary tubules constitute a mass of inextricable coils through which run the branches of the cardinal veins. Mesonephroi of the right and left sides are united in the median line to form a single compact organ. The pseudo-lymphoid tissue now forms an important constituent of the kidney. The theory of Felix that this tissue arises from the walls of the cardinal veins has already been discussed.

In the last days of embryonic life, a capsule is developed about the whole mesonephros. The flat cells composing it are of uncertain origin.

The tubules of all three orders extend a short distance posterior to the cloaca. These post-anal tubules constitute the organ described frequently as the caudal kidney.

A final component of the mesonephros remains to be described, -structures which, like the primary mesonephric tubules, develop in relation to the middle third of the pronephric ducts, but show certain peculiarities in their structure and origin which render their exact significance difficult to determine.

The anlagen of the primary tubules alternate with these "fraglichen Kanalchenanlagen". The latter contain rounded nuclei situated at regular intervals. The anlage remains solid, increases in size, and migrates toward the periphery of the mesonephros. About the time of hatching, the structures in question acquire a capsule of flattened cells, the tissue within the capsule later undergoing division into several parts. Subsequent growth results in the formation of an enormous mass of glandular appearance.

Sections through the mesonephros of a young trout of two years show typical mesonephric tubules in the median region, while almost the whole of one side is occupied by a solid glandular mass, in which no glomeruli, tubules, or duct may be observed.

In his earlier work, Felix (1897) interpreted the anlagen of this peculiar structure as the beginnings of mesonephric tubules. Swaen and Brachet (1900) objected to this interpretation and suggested that these cellular masses might be the corpuscles of Stannius. Felix (1906) gives a fuller account of the structure and development of the "fraglichen Kanalchenanlagen", and his opinion now is that the theory of Swaen and Brachet has much in its favor.

Verne (1922) recognizes in the Lophobranchs the existence of two entirely different types of structures which arise in relation to the Wolffian duct, so called because the pronephric glomerulus has already degenerated. In embryos varying in length from 15 to 20 mm, two kinds of buds appear in the region of the duct. The first arise from the duct, from which they become detached to form the corpuscles of Stannius (i.e. the cortex of the supra-renal gland. Verne here accepts the theory of Swaen and Brachet and of Audigé). The second type of anlage appears as masses situated at non - metameric intervals near the Wolffian duct, to which they become attached secondarily. These are the anlagen of true mesonephric tubules. They later become hollow, elongate, and give rise to diverticula.

The formation of Malpighian corpuscles is attributed by Verne to "une section morphogene" between the extremities of the urinary tubules and the arterial capillaries.

MATERIAL AND METHODS

The embryological series of Ictalurus punctatus which was used in this investigation was supplied by the State Fish Hatchery located at Durant, Oklahoma, through the courtesy of Superintendent J. M. Murphree. The embryos and larvae were collected at twenty-four hour intervals during the first eleven days after fertilization. The material was preserved in a mixture of glycerin and formalin of uncertain proportions. The entire series was generously placed at the author's disposal by Mr. Murphree.

The embryos were imbedded in paraffin and sectioned transversely at a thickness of ten microns. The yolk mass had hardened during fixation and in some stages constituted a serious obstacle. Early embryos were dissected off the yolk with little difficulty. The later stages were at first sectioned entire, but the large amount of brittle, unabsorbed yolk caused a tearing of the sections. Various methods were employed without success in an attempt to remedy this serious defect. Finally, the tedious and somewhat hazardous process of opening up the body wall and dissecting out the yolk under a binocular microscope was resorted to. Little or no injury resulted to the region containing the excretory organs. By this means satisfactory sections of the stages from the fifth to the ninth day, inclusive, were obtained. In later stages the small amount of yolk caused little damage when the larvae were sectioned entire.

The amount of favorable material in certain stages was limited, but in all cases it has been possible to prepare two or more satisfactory series. Two embryos of the second, tenth, and eleventh days were studied, three of the seventh and ninth, four of the fourth, fifth, and eighth, five of the sixth, and six of the third day.

Young embryos were tinged with eosin previous to imbedding to facilitate orientation, but sections were in all cases stained on the slide. Heidenhein's iron-haematoxylin followed by alcoholic eosin, and Mayer's haem-alum with the same counter-stain were both found to give good results, although in most cases a clearer picture of the renal tissue was obtained with the latter stain.

All drawings were made with the aid of a camera lucida. The photomicrographs were prepared by Mr. William L. Doyle.

THE DEVELOPMENT OF THE PRONEPHROS OF THE CATFISH

The second day.

In embryos of the second day, the mesoderm has reached a relatively high degree of differentiation. The epimere has undergone separation from mesomere and hypomere, and from the hypomere has developed a more or less distinct body cavity bounded by somatic and splanchnic epithelium.

The embryo of this stage lies above a large yolk mass. As in other Teleosts (Balfour, 1881), the endoderm forms a continuous layer beneath the splanchnic mesoderm. No space occurs between yolk and endoderm to represent the primitive gut, which is not formed as in the chick, but appears to arise as a solid cord of cells, between notochord and yolk.

The primitive body cavity or splanchnocoele in this stage then consists of two elongated spaces lying above the yolk. The coelomic cavities of the right and left sides are separated by the compact mass of relatively undifferentiated cells which is later to give rise to the alimentary canal.

The anlage of the excretory system is distinctly outlined. It may be separated into two regions: the anterior, which is to develop further into the pronephros proper (pronephric chamber and tubule), and the posterior, the pronephric or archinephric duct. The anterior region will be referred to as the pronephric chamber, following the nomenclature of Swaen and Brachet, but it must be understood that later differentiation will separate a particular part of this region as the true pronephric chamber.

The so-called pronephric chamber remains in open communication with the splanchnocoele at its medial end, while its outer end is already tubular in its nature. The orifice through which connection with the splanchnocoele is maintained might be termed a "peritoneal funnel" (Felix, Kerr) since it constitutes an opening between the pronephric chamber and the splanchnocoele. The funnel remains widely open through three sections (section thickness = 10 mu) on each side of the embryo. (Fig. 1, Pl. I) The epithelium of the

Fig. 1. Section through the pronephric anlage of a two-day embryo. N., notochord; O.E., anlage of oesophagus; P.A., pronephric anlage; P.F., peritoneal funnel; S.E., somatic epithelium; Spl., splanchnocoele; Spl.E., splanchnic epithelium. 310 X

Fig. 2. Section through the pronephric tubule of a two-day embryo. N., notochord; O.A., anlage of oesophagus; P.T., pronephric tubule. 310 X

Plate 1

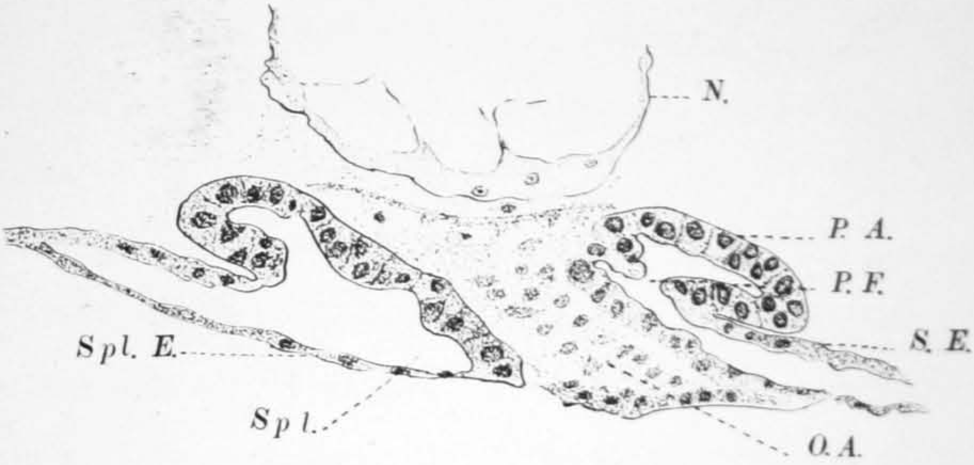


Fig. 1

Fig. 2

splanchnocoele is directly continuous through the peritoneal funnel with that of the pronephric chamber, although a gradual transition occurs from the flattened type of the former to the cuboidal epithelium of the latter.

The whole anlage of the chamber runs from the peritoneal funnel toward the lateral body wall almost directly at right angles to the long axis of the embryo, then bends slightly ventrad to communicate with the pronephric duct. The latter from this point takes a course directly caudad. The dorsal and ventral walls of the pronephric chamber, widely separated in the internal portion of the chamber, gradually converge toward the outer end. It appears that the outer portion of the chamber already constitutes a pronephric tubule which will undergo little further differentiation until after the complete formation of the true pronephric chamber and its glomus. It is impossible at this time, however, to distinguish in the median region of the chamber anlage the exact point at which the tubule begins, i.e., the nephrostome. Thr position of the pronephric tubule in the mesenchyme lateral to the splanchnocoele is such that its cells are in contact with the somatic epithelium (lining the coelom). This relation is also true of the pronephric duct at its anterior end. (Fig. 2, Pl. I; Fig. 3, Pl.II.)

It should be noted here that the portion of the splanchnocoele which lies nearest the median axis of the embryo is a clearly defined cavity. Into this region the peritoneal funnel opens.

Toward the outer limits of the embryo the coelomic cavity narrows to a mere slit, (sometimes almost obliterated), between somatopleure and splanchnopleure. In some sections there is a strong suggestion of a fusion between somatic and splanchnic epithelium, separating the splanchnocoele into two distinct regions. (Fig. 2, Pl.I)

During the course of development, with the formation of the alimentary canal in the region now directly ventral to the notochord, the configuration of the coelomic cavities will be altered, so that the right dorsal body cavity will be separated from the left only by the dorsal mesentery. The intestine anlage, suspended in the splanchnocoele, will then occupy a position distinctly ventral to the anlage of the pronephric chamber, although in the stage under discussion the two lie in the same horizontal plane.

In embryos of the second day, the pronephros proper lies adjacent to the dorso-lateral wall of the splanchnocoele. The development of the alimentary canal and its subsequent isolation in the splanchnocoele are accompanied by a general migration of other structures toward the median line, so that when the pronephric chamber is encountered in the next stage, it will be found directly above the splanchnocoele and medial to its present position.

The pronephric ducts in this state are completely isolated from the splanchnocoele, from the walls of which, it may be supposed, they have arisen. As they run caudad from their point of communication with the pronephric tubule, the ducts migrate ventro-laterally

Fig. 3. Section through the pronephric duct of a two-day embryo. I.A., intestine anlage; N., notochord; P.D., pronephric duct; Spl., splanchnocoele. 310 X

Fig. 4. Section through the pronephric ducts in the posterior region of a two-day embryo. Lettering as above. 310 X

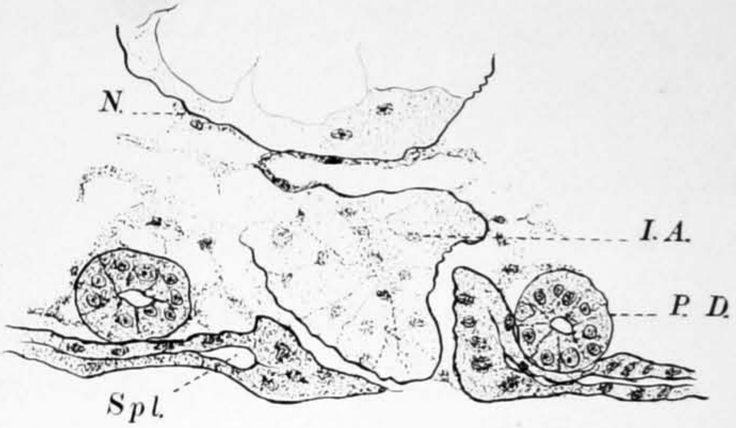


Fig. 3

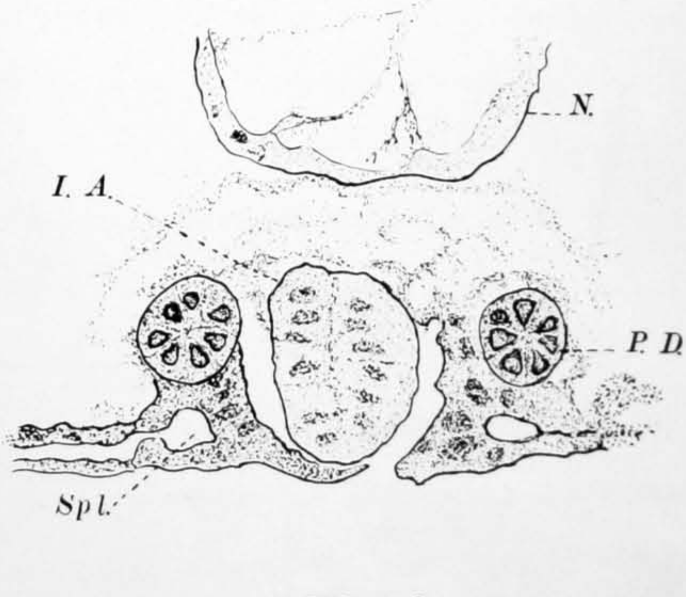


Fig. 4

and acquire the position indicated. (Fig.3, Pl.II) The lumen of the duct is distinct throughout its anterior region, but is frequently undeveloped toward the posterior. This fact may indicate that the duct develops as a solid cord of cells which acquires a lumen secondarily. The radial disposition of the cells about a central axis is preserved consistently, however, except in the extreme caudal region, where the cells are distinguished from the surrounding mesoderm only with difficulty.

The principal cavity of the splanchnocoele is greatly reduced a short distance posterior to the pronephric tubule. The exact means by which this is accomplished is not clear, but there are indications that the reduction is effected by the gradual approach of the somatic and splanchnic walls of the splanchnocoele, their ultimate contact and consequent reduction of the intervening space to a mere slit. (Fig. 3, Pl. II) Eventually only a small round space directly ventral to each duct represents the splanchnocoele. (Fig. 4, Pl.II) These relations are maintained with very little change for a considerable distance.

The embryo then begins to increase in depth toward the posterior end, and with this change the pronephric ducts gradually migrate to a position rather more dorsal than lateral to the gut, and thus approach each other until their epithelial walls are finally in contact.

In this region a structure strongly resembling the dorsal aorta occurs ventral to the notochord. It cannot be traced into the anterior regions, and the general relations of the circulatory system in this stage are not readily ascertained from the material at hand.

Gut and ducts gradually become less distinct, until their cellular boundaries are broken down and the whole becomes fused into an indistinguishable cell mass. The pronephric ducts do not communicate with the exterior in this stage.

The third day.

The observations made on embryos of the third day have necessarily been of a somewhat fragmentary and unsatisfactory nature. Fixation of tissues in embryos of this stage was uniformly poor. The embryos have undergone considerable elongation and have coiled about the yolk in such a way that when detached from the latter, the embryo is in the form of an almost complete ring. For this reason it has been necessary to cut each specimen into several species, which were sectioned separately. Even by this means, it has not been possible to cut perfect transverse sections in every region of the embryo. While structures in certain restricted areas may be followed through the series with accuracy it is impossible to determine the formation of others. Fortunately, the region of the pronephric chamber is intact in several series. Here the general relations may be ascertained, although the poor fixation renders a completely satisfactory account impossible.

Differentiation of the anterior end of the pronephros has progressed rapidly. The internal end of the anlage, no longer in communication with the splanchnocoele through the peritoneal funnel, has expanded into a rounded vesicle, the true pronephric chamber. In some embryos, the chamber is almost circular in cross section, while in others it retains its more primitive elongate shape. These variations in form are portrayed in Figures 5 and 6, Pl.III)

The external half of the pronephric chamber anlage, which twenty-four hours earlier was recognizable as a pronephric tubule, is now sharply separated from the chamber. A true nephrostome, or opening from tubule to pronephric chamber (thought to be originally a part of the splanchnocoele) is apparent.

The most important advance seen in embryos of the third day is the establishment of relations to the circulatory system through the formation of the glomus. The pronephric chambers of the right and left sides have approached each other in the median line, but are still separated by an appreciable width of mesoderm. The inner wall of each chamber is, according to Swaen and Brachet, composed of splanchnic mesoderm. (This fact could not be verified in the present investigation, due to the lack of earlier stages.) In each embryo of this age studied, the medial, or dorso-medial splanchnic wall of the chamber is greatly evaginated to accommodate a bulging mass of tissue which fills the projection into the chamber. This tissue is evidently the glomus of the pronephros.

Several variations in the form of the glomar evaginations have been observed. These are indicated in Figures 5 to 7 , Pl.III)

Fig. 5. Section through the anlage of the pronephric chamber and glomus of a third-day embryo. Ao., aorta; D.H., dorsal horn of pronephric chamber; Gl., glomus; Ne., nephrostome; O., oesophagus; P.T., pronephric tubule; S.E., somatic epithelium; V.H., ventral horn of pronephric chamber. 330 X

Fig. 6. Section through the same region of another embryo of the third day. Lettering as above. P.C., pronephric chamber. 330 X

Fig. 7. Section through the same region of another embryo of the third day. Lettering as above. 330 X

Plate III

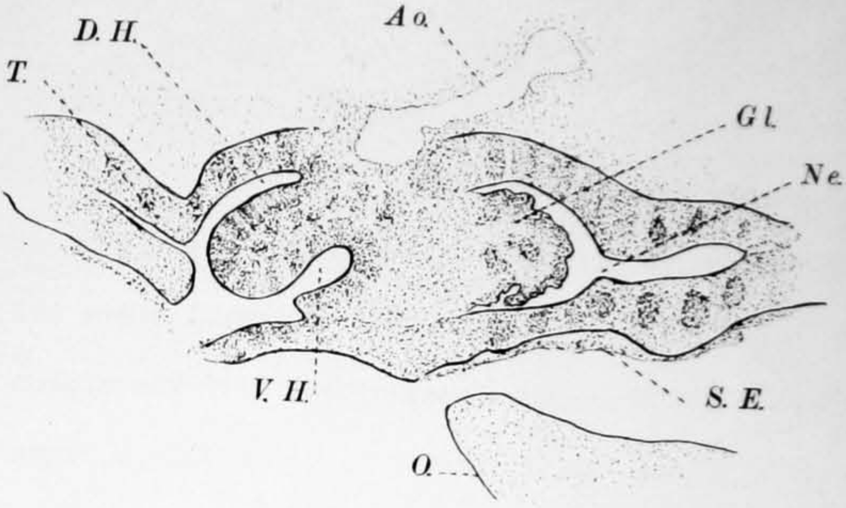


Fig. 5

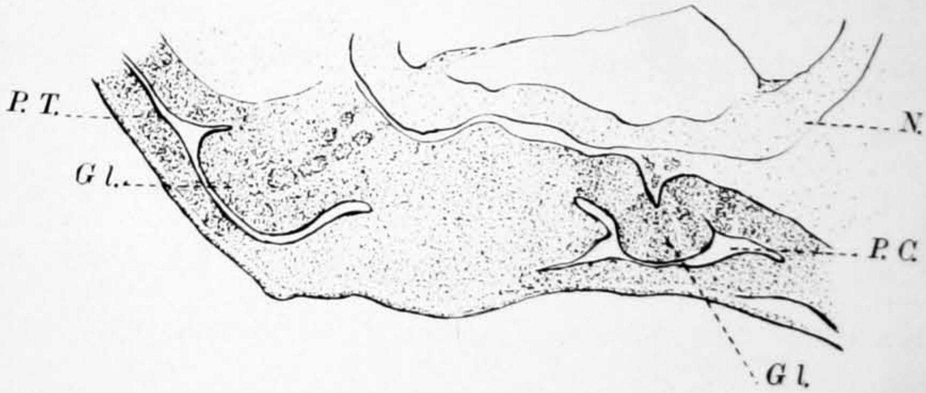


Fig. 6

Fig. 7

In some cases the glomus projects at right angles to the internal wall of the chamber, midway between its dorsal and ventral boundaries. (Fig. 5) In others, (Fig. 6), the glomus is suspended obliquely in the chamber, from the angle formed by its dorsal and medial walls. The glomus may almost completely fill the internal region of the chamber. In these cases, a small slit-like prolongation of the chamber slips between the glomus and the ventral wall toward the median line. (Probably this represents the "ventral horn" of Felix.) (Fig. 7, Pl. III)

The segmental tubule of the pronephros communicates with the pronephric duct, which runs posteriad dorsal to the developing intestine. The caudal ends of the ducts appear to fuse with the wall of the gut. An opening occurs in one series, immediately posterior to this fusion, but whether it represents the external aperture of the intestine and excretory ducts, or is merely a tear in the section cannot be determined in this material. From the fact that no opening occurs in the fourth day, it is considered probable that the seeming orifice is a defect in the section.

The fourth day

The excretory organs in embryos of the fourth day present the essential characteristics of a typical Teleostean pronephros.

Relations with the arterial system are well established by the further development of the glomus, which first appeared on the third day. (Fig. 8, Pl. IV)

The dorsal and ventral "horns" of each chamber now pass above and below the glomus, each almost meeting in the median line with the corresponding structure of the opposite side. This process results in the formation of a median septum which separates the pronephric chambers of the right and left sides.

The epithelium lining the pronephric chamber has undergone a change from the characteristic cuboidal type which characterized the epithelium of the undifferentiated chamber in earlier stages, to a thin flattened epithelium. It might be expected that the glomar tissue, in pushing into the pronephric chamber, would have carried the splanchnic lining before it. There is no indication that the glomus is covered by epithelial cells. Its extremely irregular outline is suggestive of a cluster of grapes, and apparently the rounded, deeply-stained cells which are scattered throughout the glomar tissue project freely at its periphery into the pronephric chamber.

The cells referred to appear to be embedded in a finely granular tissue, which is traversed by numerous capillaries. (Fig.8, Pl.IV)

In embryos of the fourth day, the glomus is a-symmetrical at its extreme anterior end, appearing first on the right side of the embryo. The glomus of the left side appears in the next section, and here the two chambers are separated by the median septum, which is heavy in the anterior end. It evidently represents the remnant of the strip of mesoderm which in earlier stages separated the chambers of the right and left sides.

It might be anticipated from the mode of development of the glomus that the original bi-lateral symmetry of the structure would be retained to the extent that the glomar tissues of the right and left sides would be distinguishable. There are indications, however, that a thinning of the intervening septum occurs in the region half way between the anterior and posterior limits of the chambers, with the fusion of the glomi of right and left sides. This fusion may not be complete. In some sections the septum, which appears to suspend the glomar tissue in the chamber, seems to be continued through the glomi from mid-dorsal to mid-ventral wall of the chamber, separating the vascular mass into two parts. (Fig. 8 Pl.IV) In others, the capillary spaces of the glomi are distributed throughout the whole structure, and traces of a septum are distinguishable. (Fig.9, Pl.IV) The glomar mass, although undoubtedly it originated from two widely separated and bi-laterally symmetrical vascular strands, is now to all appearances united into a single median structure. The term "glomus" will hereafter be applied to the whole mass of vascular tissue, although it is recognized that it arises from two distinct glomi. Similarly, the term "pronephric chamber" will be used to represent the entire space in the median line of the embryo, originally derived from two widely separated chambers.

In one series, the pronephric chamber appears to communicate with the splanchnocoele near its anterior end on the right side. This fact could not be confirmed in any other series of this stage.

Fig. 8. Section through the pronephric chamber and glomus of a fourth day embryo. Ao., aorta; C., capillary; D.M., dorsal mesentery; Gl., glomus; O., oesophagus; P.C., pronephric chamber; P.T., pronephric tubule; S., septum; Spl., splanchnocoele. 380 X

Fig. 9. Section through the pronephric chamber and nephrostome of the left pronephric tubule of a fourth day embryo. Lettering as above. Ne., nephrostome. 380 X

Fig. 10. Section through the posterior region of a four-day embryo, showing the anlagen of the supposed corpuscles of Stannius. C.S., corpuscles of Stannius; C.V.A., anlage of cardinal veins; P.D., pronephric duct. 380 X

Plate IV

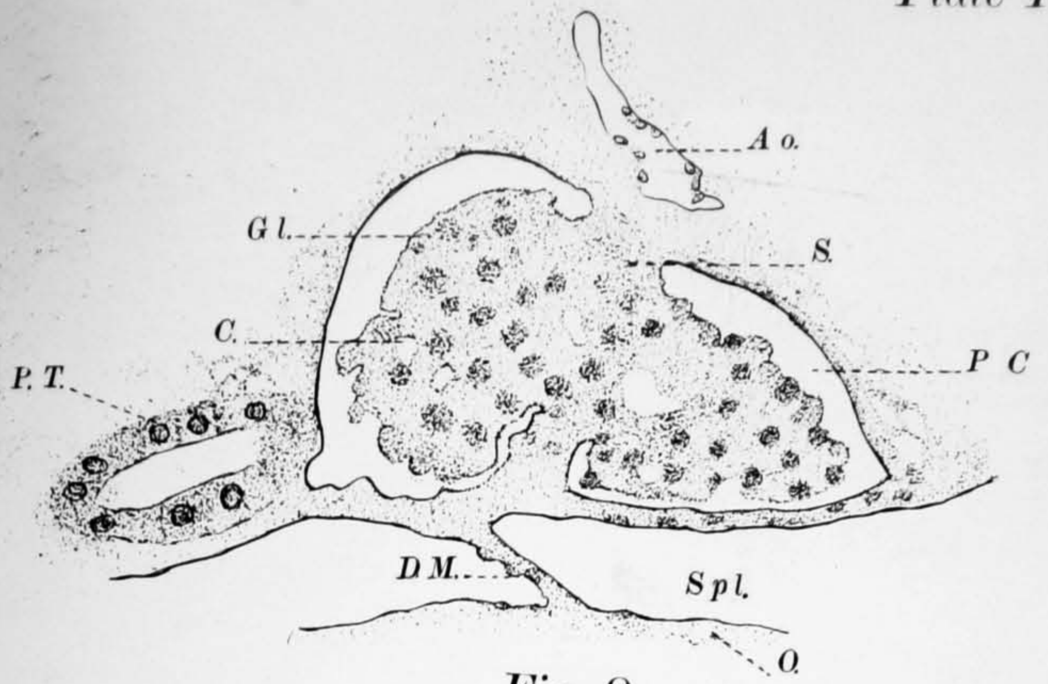


Fig. 8

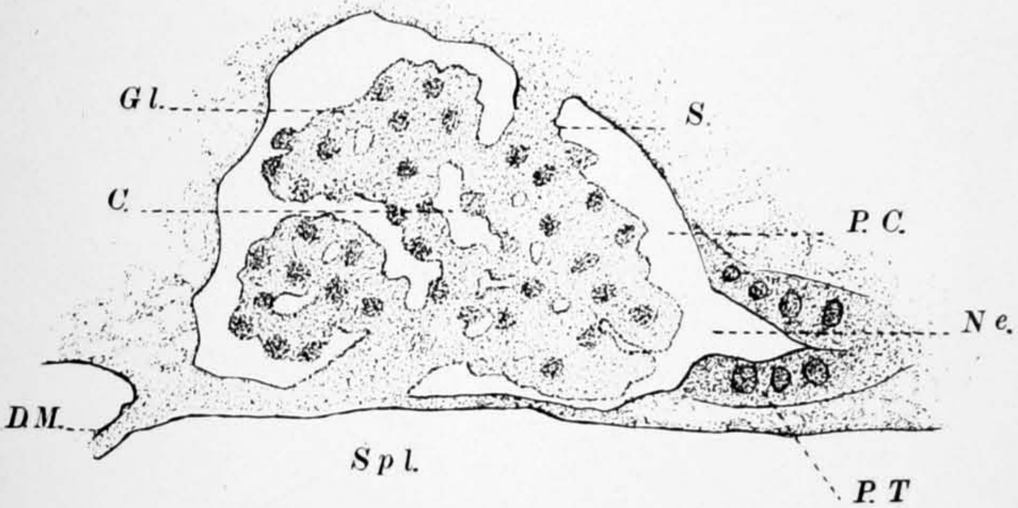
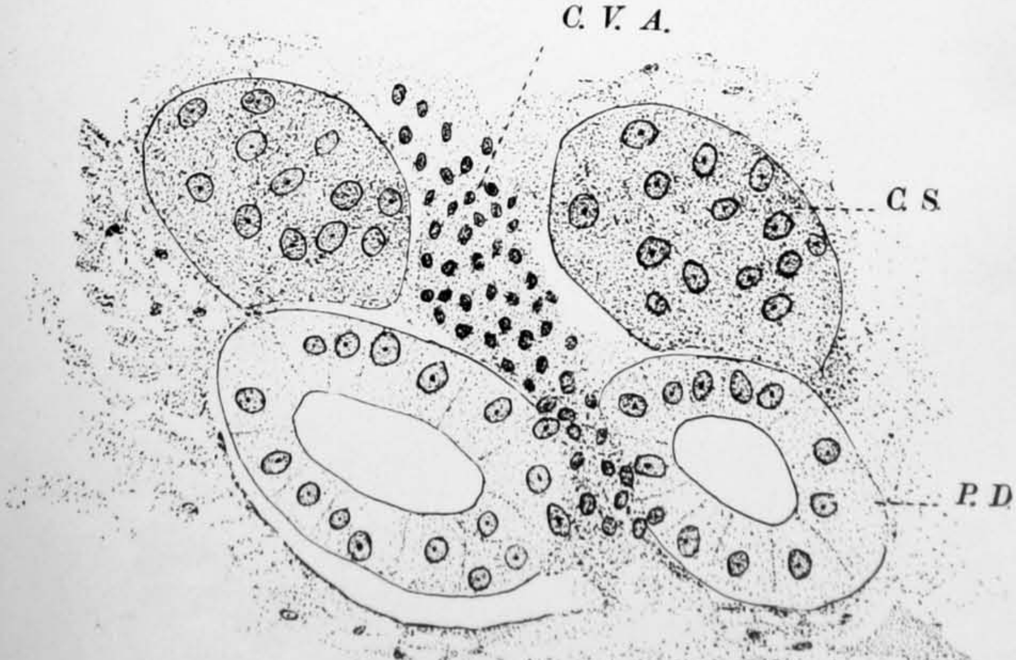


Fig. 9



If such a connection actually occurs, it may indicate merely that the complete separation of the two which will later occur has not been consummated at this stage of development.

The pronephric tubule opens into the chamber through a nephrostome directly opposite the glomus. The nephrostome of the right side appears slightly anterior to that of the left, and indeed is closed off completely before the latter makes its appearance. (This anterior extension of structures of the right side has been noted in the majority of the sections cut. It was at first attributed to the possibility that the sections were not perfectly transverse, but the regularity with which the phenomenon occurs indicates that this slight a-symmetry may actually be of frequent occurrence.)

The tubule is lined with typical cuboidal epithelium, with distinct, rounded nuclei. The tubule of the right side runs almost directly laterad and slightly posteriad above the splanchnocoele, in each of the four embryos studied. When it reaches the position indicated in Fig.8, Pl.IV), it communicates with the pronephric duct. The point at which the tubule opens into the duct is four sections posterior to the nephrostome. It is thus apparent that the pronephric tubule in this stage is a simple, uncoiled structure, its course from nephrostome to duct being almost directly laterad.

The nephrostome of the left side appears at the same transverse level as the duct of the right. It is widely open to the pronephric chamber, its epithelium undergoing gradual transition into that of the latter. The orifice of the nephrostome is filled by a substance of shadowy appearance which may be cilia, but could not be positively identified as such even under oil immersion. The tubule of the left side takes a course somewhat more directly posteriad than laterad. (This condition occurs definitely in two of the four embryos sectioned. Defects on the left side of the other two embryos render the course of the tubule doubtful in these cases.) The left tubule communicates with a duct which lies in the same position with respect to the splanchnocoele and oesophagus as the duct of the right side. (Fig. 11 Pl. V) The component cells of the pronephric ducts are radially disposed around a central lumen.

As the pronephric ducts pass caudad through the embryo, they converge gradually toward the median line, in conformity with a change in the shape of the splanchnocoele. The epithelium of the ducts is in contact with the somatic epithelium. The ducts occupy a position adjacent to the region where the main cavity of the splanchnocoele (surrounding the alimentary canal), leads into its long, lateral extensions over the yolk mass. As the central part of the splanchnocoele decreases in width, the ducts migrate to a position nearer the median line. Figs. 11, 12, Pl. V)

The ducts are ultimately separated by the width of only a few cells, and at times they appear to be in contact. In this position they pursue a course dorsal to the intestine. Occasional changes in the diameter and position of each duct indicate that their course is slightly irregular.

At a considerable distance posterior to the pronephric chamber, solid cell masses appear in the mesenchyme in continuity with the dorsal surface of the pronephric duct. (Fig.10,Pl.IV) These structures persist through five sections on the right side and four on the left. Their occurrence has been confirmed in several series of the four day embryo.

These cell masses are similar to the structures described by Felix (1906), and interpreted by him and by Swaen and Brachet (1900) as the probable anlagen of the corpuscles of Stannius.

The appearance of each anlage is that of a rounded mass with a concavity in its ventral surface where it rests against the duct. Cell walls cannot be distinguished, but as many as fourteen nuclei have been counted in a single cross section through one anlage. The nuclei resemble those of the duct epithelium, while the cytoplasm of the structure in question stains much more deeply than that of the cells composing the duct.

The same difficulty which was indicated above was experienced in sectioning the embryos of this stage, because of their coiled position around the yolk. For this reason, it cannot be stated positively that the ducts communicate with the exterior, although there are indications in several embryos that this is the case. The ducts appear to unite and to run ventrally around the intestine. (Fig. 13,14, 15, Pl. V) Such a fusion of the excre-

Fig. 11. Diagrammatic representation of a section through the pronephric ducts posterior to the pronephric chamber in an embryo of the fourth day. A.C., alimentary canal; P.D., pronephric duct; Y., yolk. 150 X

Fig. 12. Section through the same embryo, at a more posterior level. Lettering as above. 150 X

Figs. 13 - 17. Successive sections through the posterior ends of the pronephric ducts of a fourth day embryo. I., intestine; P.D., pronephric duct; U.B., urinary bladder. 150 X

Plate V

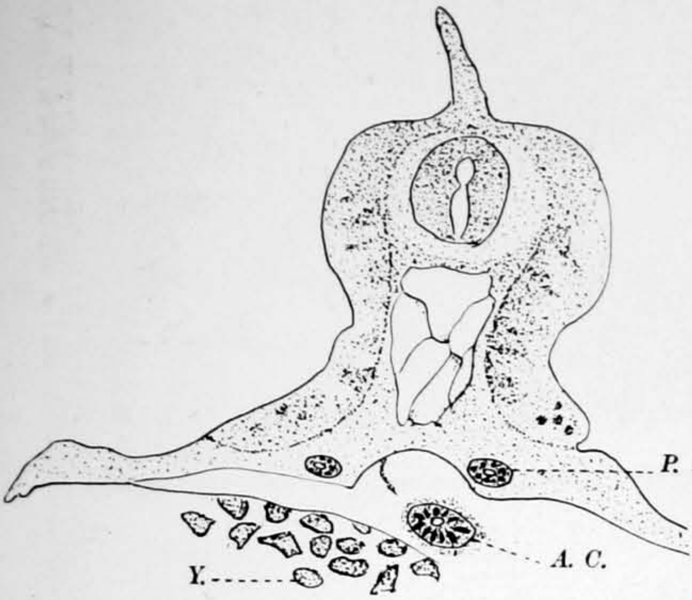


Fig. 11

Fig. 12

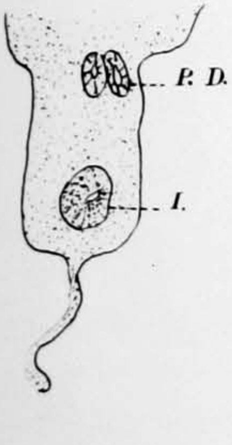


Fig. 13

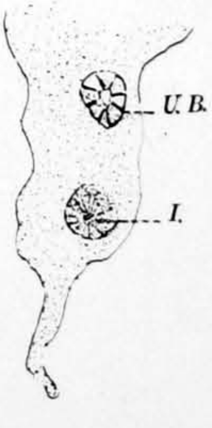


Fig. 14

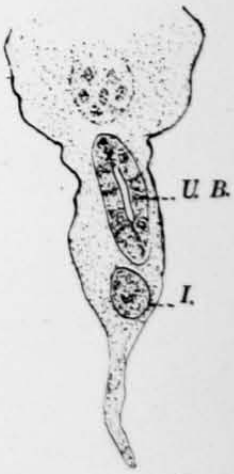


Fig. 15

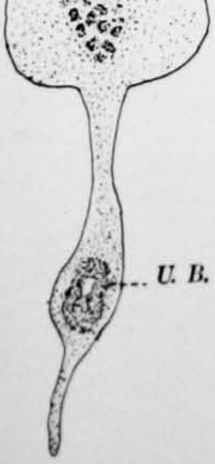


Fig. 16

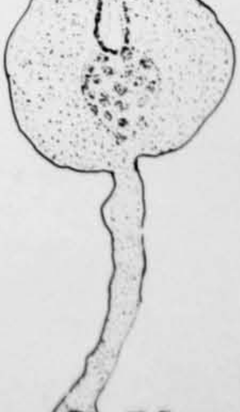


Fig. 17

tory ducts is common in Teleosts, and the structure thus formed is called the "urinary bladder". It is impossible to determine from this material whether the intestine ends blindly here, (Fig. 15, Pl. V), the fused urinary ducts running slightly posterior and ventral to it, (Fig. 16 Pl. V) or whether they open into the intestine, forming a sort of cloaca. C. K. Hoffmann (1886), describes the union of the posterior ends of the segmental ducts of Teleosts into an unpaired tube, the "so-called urinary bladder", which opens dorsally into the intestine: "bei jungen Embryonen ist also eine Kloake vorhanden, obgleich dieselbe dann noch nicht mit der ausseren Welt kommunicirt." This appears to be in agreement with the condition found in the catfish on the fourth day. No actual opening was found, but the lining of the "cloaca" or the "urinary bladder", as the case may be, is apparently in contact with the ectoderm. (Fig. 17, Pl. V)

The fifth day

The excretory organs of embryos of this stage show an advance over those of the fourth day only in the increased complexity of structures already formed.

The glomus has increased in size until it almost completely fills the pronephric chamber. (Fig. 18, Pl. VI) At its anterior end masses of glomar tissue extend to left and right from a median septum, but in all other sections, the whole is fused into a structure the double nature of which is indistinguishable. The spaces representing capillary vessels are scattered throughout the glomus,

Fig. 18. Section through the pronephric chamber and tubule of a fifth day embryo. Ao., aorta; D.M., dorsal mesentery; Gl., glomus; Ne., nephrostome; P.C., pronephric chamber; P.D., pronephric duct; P.T., pronephric tubule; Spl., splanchnocoele. 380 X

Fig. 19. Section through the same embryo, at a slightly more posterior level. Lettering as above. C.V.A., cardinal vein anlage. 380 X

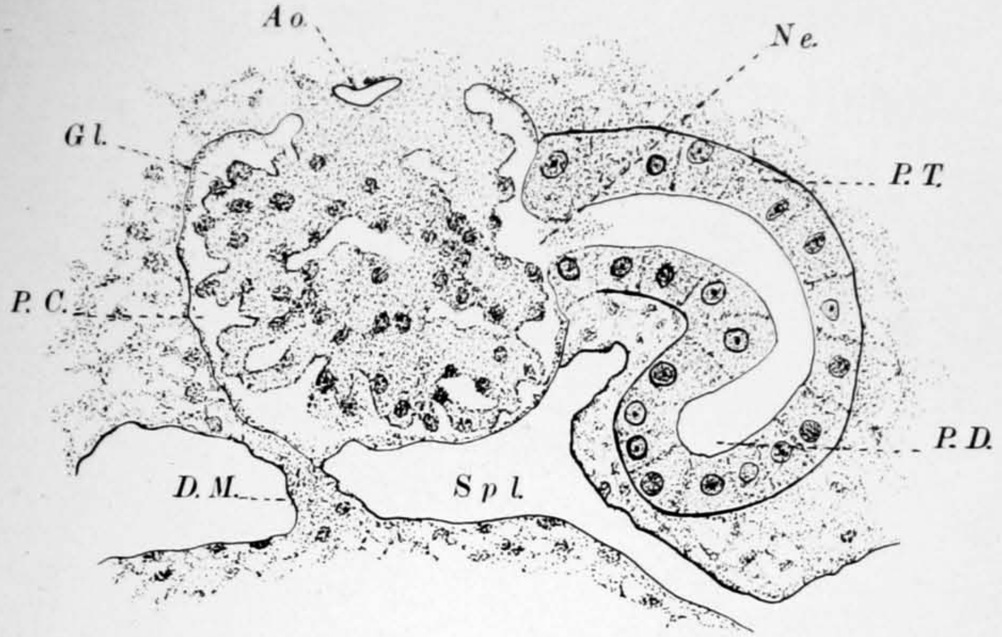


Fig. 18

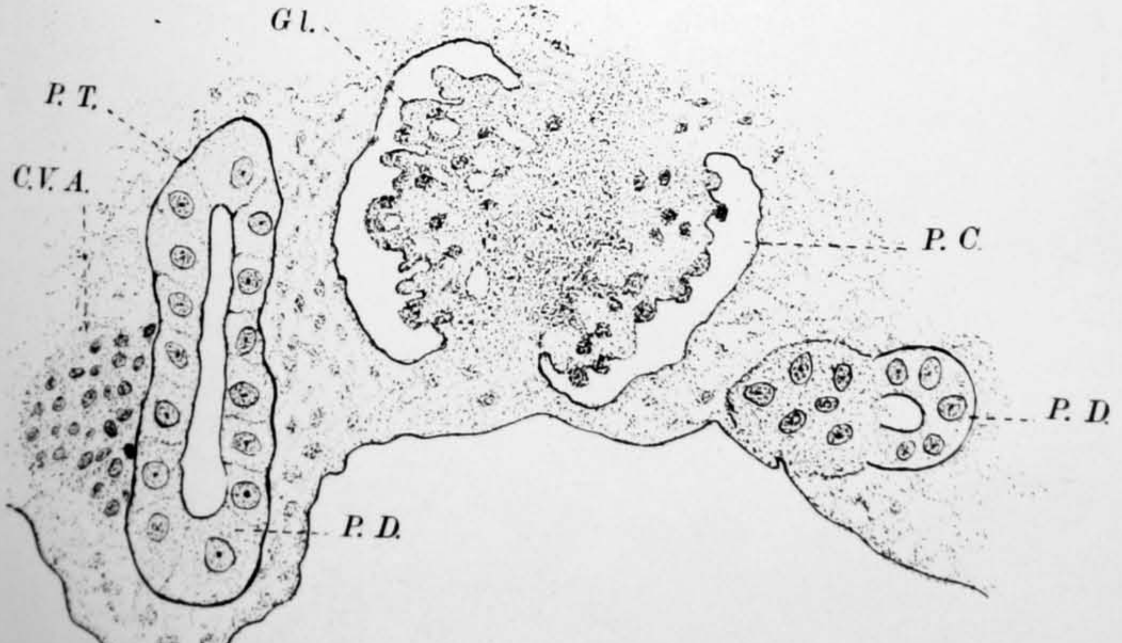


Fig. 19

which lies directly ventral to the aorta.

The epithelial cells of the pronephric chamber are of the flattened type as before.

The nephrostomes of right and left pronephric tubules lie almost directly opposite each other. Nephrostome and duct on the left side are in the same transverse plane, the tubule describing a curved path laterally, ventrally and back toward the median line, but not running posteriorly, as was the case in the four day embryo described. (Fig. 18, Pl. VI) The duct of the right side is formed close to the ventro-lateral wall of the pronephric chamber, but runs a short distance laterally through the undifferentiated mesoderm, until it reaches a point directly under the scleromyotome, where it turns and takes up its posterior course. The tubule of the right side appears to run ventrally and posteriorly to its duct. (Fig. 19, Pl. VI)

The pronephric ducts migrate toward the median line in the manner described in the preceding stage, and take up a position dorsal to the intestine. (Fig. 20, Pl. VII) They proceed posteriad by a somewhat irregular course, looping dorsally and ventrally along a straight longitudinal axis.

The supposed anlagen of the corpuscles of Stannius first described in the embryo of the fourth day have undergone no further apparent differentiation.. They remain as a pair of solid cell masses, one dorsal to each duct.

Communication with the exterior has been established in

Figs. 20-26. Successive sections through a fifth-day embryo in the region of the urinary bladder. I., intestine; P.D., pronephric duct; U.B., urinary bladder. 150 X

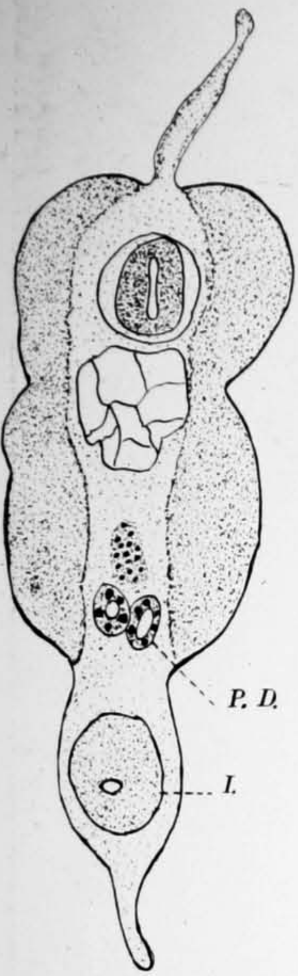


Fig. 20

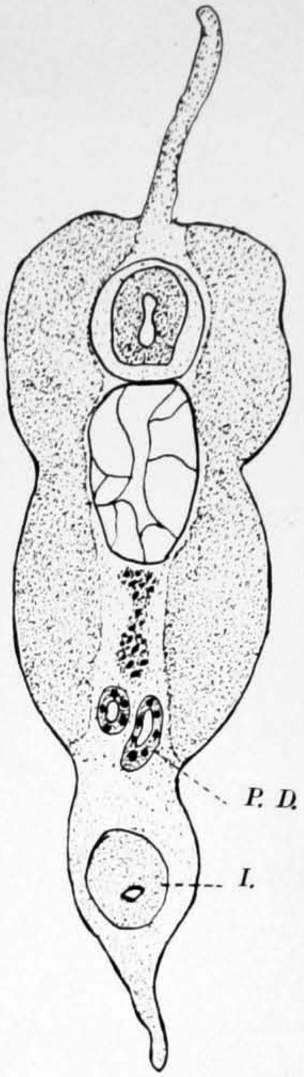


Fig. 21

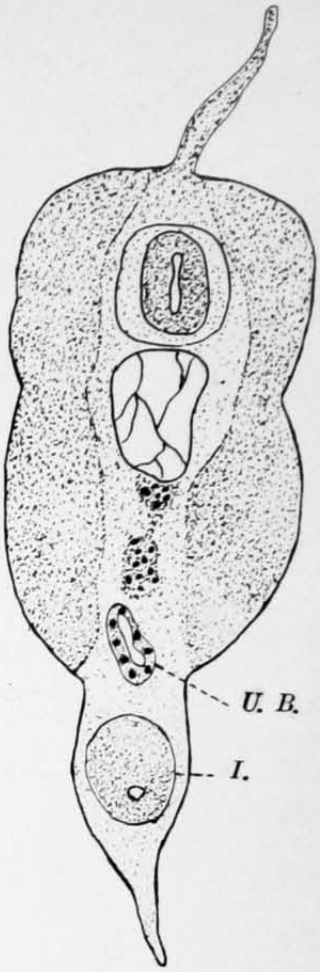


Fig. 22

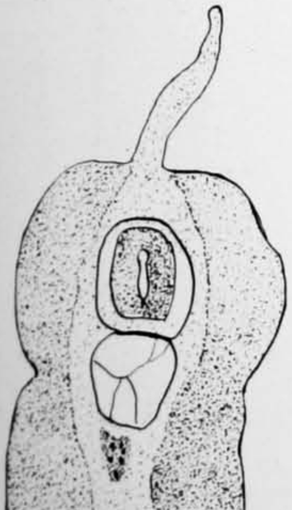
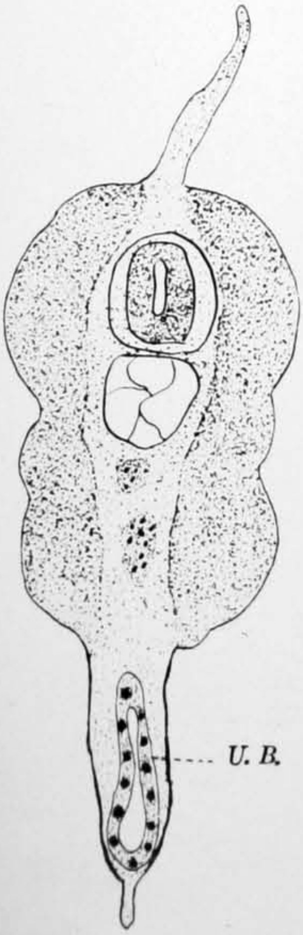
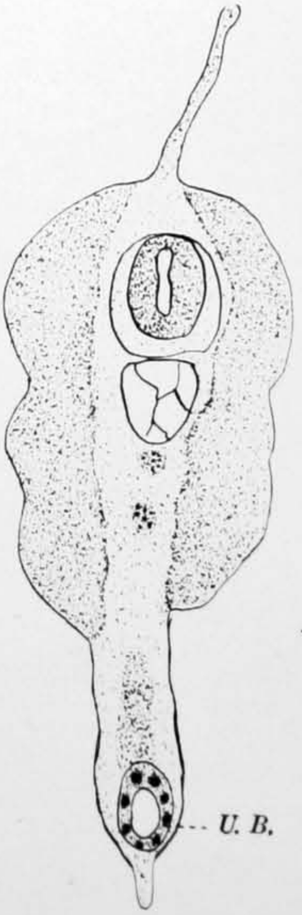
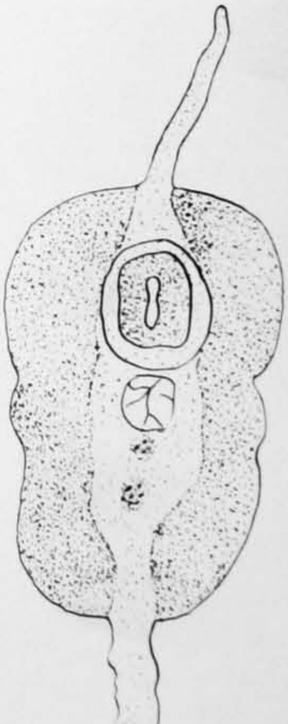


Fig. 23







this stage. In the posterior body region, fusion of the two pronephric ducts occurs. (Fig.22, Pl.VII) The "urinary bladder" thus formed runs ventrally and approaches the intestine, its cells coming into actual contact with those of the latter. The intestine here appears as a solid mass, and the lumen of the bladder seems to sink into it. (Fig.23, Pl.VII) Two interpretations appear possible. There is some slight evidence that the lumen of the "bladder" may become continuous with the intestine, the two having a common opening. The intestine, on the other hand, may end blindly in a solid cord of cells with the "urinary bladder" skirting its posterior margin and opening independently. The fact that the intestine loses its lumen before that of the "bladder" sinks into it appears to support the latter theory. These relations are indicated in Figures 23 - 26, Pl.VII) Felix (1897) describes an apparently similar condition in the trout and salmon, where the intestine becomes separated from the bladder, its blind end remaining in contact with the wall of the latter. The "rectum" later breaks through as an independent opening anterior to the "Orificium externum urethrae."

The sixth day

Significant changes are now initiated in the region of the pronephros.

The pronephric tubule no longer runs freely through the mesenchyme, but is surrounded by a mass of rounded, closely packed cells which now makes its appearance for the first time. This is probably the anlage of the tissue described in the literature as the "pseudolymphoid" or "lymphoid" tissue characteristic of the Teleostean pronephros.

Since in the embryonic or larval stages at least, there is demonstrable in this tissue nothing of the reticular organization which the term "lymphoid" connotes, the objection of Felix to the use of this adjective is considered valid, and the word "pseudolymphoid" suggested by him is tentatively adopted.

The cells of the pseudolymphoid tissue are confined within a space of regular outlines which extends across the mid-line of the embryo, beneath the notochord, its continuity interrupted only by the pronephric chamber. The pronephroi of the opposite sides have thus become united in a compact organ of elongate shape, consisting of two distinct elements: the renal elements proper (tubules, glomus,) and the pseudolymphoid tissue. (Fig.51, Pl.XI) The present investigation throws no light on the origin of the pseudolymphoid tissue, which appears suddenly on the sixth day. No elements found in the five-day embryos can be said with certainty to have given rise to it, although it appears to sustain a relation of possible significance (which will be discussed elsewhere) to the developing vascular tissue. A fuller investigation of this question is contemplated.

The single pronephric tubule is now coiled, instead of running directly from the nephrostome to the pronephric duct. The nephrostomes are much less distinct in this series than in earlier stages.

The glomus has increased in size until its peripheral cells are almost in contact with the lining of the pronephric chamber, and the heavily staining pseudolymphoid cells crowd so closely upon the cells of the tubule that it is difficult to make out the exact position of the nephrostome on either side. (Fig.27,Pl.VIII) From the location of the tubules with respect to the wall of the pronephric chamber, the approximate position may be determined. It appears that the tubules turn anteriorly from the nephrostomes, running in this direction through four sections on the right side and three on the left. Each then turns laterad, and loops directly back on its course. The posteriorly directed loop runs at first at a slightly more dorsal plane than the anteriorly directed. The result of the course described is obviously that a much larger amount of tissue is traversed and drained by the tubules than was the case in earlier stages. Here is the beginning of a transition toward the stage of much greater physiological efficiency which appears on the eighth day, where the tubules ramify throughout the whole pronephric tissue.

The pronephric chamber extends through six sections, instead of ten as in the five-day embryos. There is no indication of any communication with the splanchnocoele.

A long space intervenes between pronephros and mesonephros, and is traversed by the pronephric ducts alone. For convenience, this region will be designated as the inter-nephric space. It corresponds to the interval occupied in older embryos by the air-bladder, which has not yet appeared. A similar interval was described by Emery (1882) for Zoarces, Belone, and Fierasfer. He refers to the portion of the renal duct which unites pronephros and mesonephros as the "isthmus".

It will be recalled that the only structures which were found on the fifth day in the region of the future mesonephros consisted of a pair of solid anlagen continuous with the epithelium of the pronephric duct. These were interpreted tentatively as the corpuscles of Stannius.

The anlagen of primary mesonephric tubules as described by Felix were not found in this stage. In the mesonephric region of the sixth day, however, the ducts receive relatively simple tubules, the coils of which, imbedded in the round-celled pseudolymphoid tissue, occupy most of the space of the mesonephros. (Fig.30, Pl.VIII) The loops of the tubules appear in general to extend in a dorso-ventral direction. The development of the anlagen from which these short tubules are derived has evidently taken place during the twenty-four hour interval between the fifth and sixth days.

The anterior end of the mesonephros is clearly in a more advanced state of development than the posterior. In relation to the anterior end of the duct (which in this region may now be termed the "Wolffian duct",) there appear the simple mesonephric tubules previously described and figured. The posterior mesonephric region contains no such structures, but here are found solid clusters of cells

Fig. 27. Section through the pronephric tubule of a sixth day larva, showing the anlagen of the cardinal veins and of the pseudolymphoid tissue. C.V.A., cardinal vein anlage; Gl. glomus; L.T., pseudolymphoid tissue; P.C., pronephric chamber; P.T., pronephric tubule. 380 X

Fig. 28. Section through the anlage of a mesonephric tubule on the sixth day. M.D., mesonephric duct; M.T.A., mesonephric tubule anlage. 380 X

Fig. 29. Section through the anlagen of a mesonephric tubule and the Corpuscles of Stannius of a sixth day larva. Lettering as above. C.S., corpuscles of Stannius. 380 X

Fig. 30. Section through the mesonephros of a sixth day larva. C.V.A., cardinal vein anlage; L.T., pseudolymphoid tissue; M.D., mesonephric duct; M.T., mesonephric tubule; N., notochord. 380 X

Plate VIII

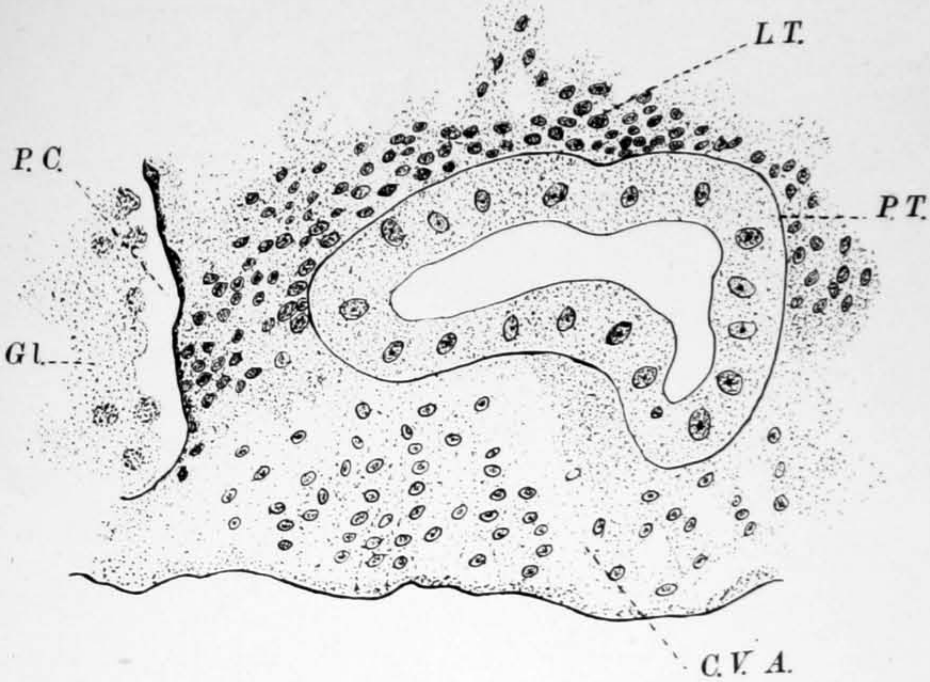


Fig. 27

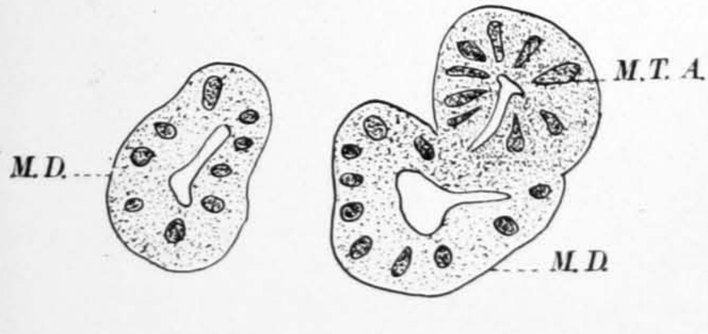


Fig. 28

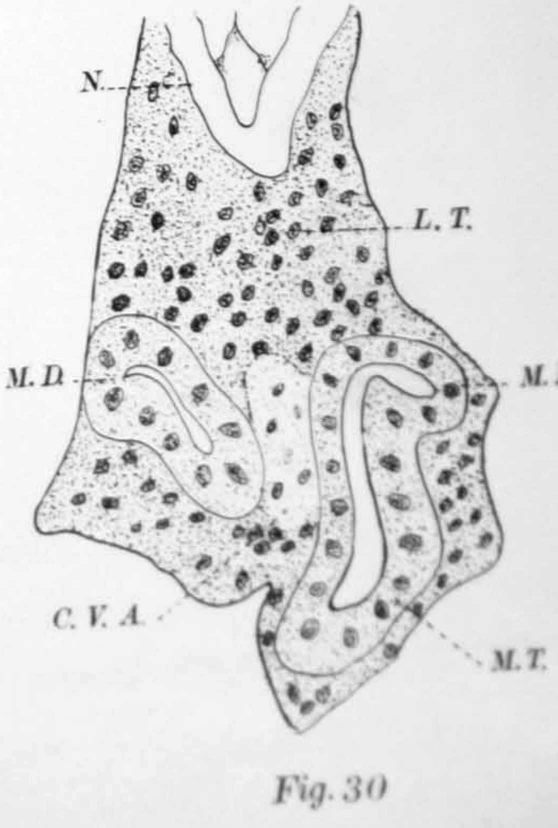


Fig. 30

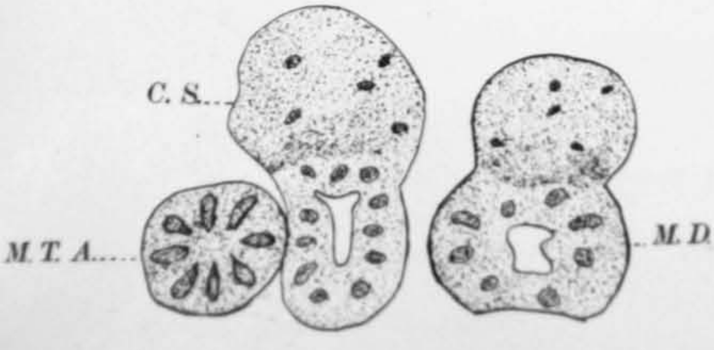


Fig. 29

in contact with the duct epithelium. These may represent the anlagen of mesonephric tubules. Due to the extremely rapid development of the catfish, a series of embryos only a few hours apart would be necessary for a thorough study of these structures. With the evidence at hand, it cannot be determined whether these tubules are primary or secondary, according to the classification of Felix, although it is thought that the tertiary tubules may be excluded from consideration because of their later origin.

It was hoped that this posterior, undifferentiated region might show some transitional stages between the solid cell mass stage and the open tubules of the anterior mesonephros. Only one or two indications of intermediate structures were found.

In one section, the tubule anlage contains a lumen which extends toward, but does not penetrate, the duct epithelium. (Fig.28, Pl.VIII)

In others, the cells of the anlage show a tendency toward radial disposition around a central axis, the place of the future lumen. (Fig.29, Pl.VIII)

In still another section, there are indications that the anlage may be slightly coiled while still in the solid condition.

The mesonephros has at this time no connection with the arterial system by means of glomeruli. Blood-filled spaces, however, indicate the beginning of the intricate venous system whose capillaries and sinuses traverse the Teleostean mesonephros.

The antero-posterior extent of the mesonephros is much greater than that of the pronephros, Computations based upon the number of sections occupied by each structure indicate that eleven per cent of the space from the anterior tip of the pronephros to the last mesonephric tubule anlage, is occupied by the pronephros; fifteen per cent comprises the inter-nephric space, while the mesonephros constitutes the remaining seventy-four per cent.

The anterior, relatively well-differentiated part of the mesonephros, occupies approximately a third of the total mesonephric space.

Opening of the ducts to the exterior is clearly established. There are indications here that the intestine now utilizes the urinary orifice. No union of the intestine and bladder occurs, however, the intestine lying a considerable distance ventral to the bladder. (Fig. 36 Pl. X) The urinary bladder begins to run ventrally (Fig. 37, Pl.X), and approaches, but never comes into contact with, the intestine. The latter communicates with the exterior (Fig.38, Pl.X) and the bladder, running caudad, comes to lie posterior to the end of the intestine, and opens through the same orifice. This opening is interpreted as actually that of the excretory system, which is temporarily utilized by the intestine, for the reason that a urinary aperture, with similar relations to the bladder, occurred on the fifth day, at which time the intestine ended blindly. The details of the formation of the anal opening in the cat fish thus appear to differ from the condition described by Felix (1897) for the trout and salmon. (Fig.38,39 Pl. X)

The excretory system at this time will be seen to be potentially one of considerable physiological efficiency. The changes described may be correlated logically with the fact that on the sixth day the embryo escapes from the investing egg membranes, and becomes a larval fish.

The seventh day

The pronephros of the seventh day shows little advancement in development over the preceding stage. The anterior end is practically unchanged. The round-celled packing tissue of the kidney, or "pseudolymphoid tissue", is excessively abundant.

The anterior tips of the right and left pronephroi are widely separated by the round-celled cartilage investing the notochord. The diameter of the mass of cartilage decreases posteriorly, and a thin strand of pseudolymphoid cells slips under it to approach the aorta in the median line. This median migration of pseudolymphoid cells takes place slightly anterior to the pronephric chamber. The pronephros now becomes a single unpaired structure, its right and left halves united in the median line by a bridge of pseudolymphoid tissue in which is situated the pronephric chamber. Its dorsal surface still shows a deep concavity where it lies upon the notochord and its investing cartilage.

The glomus begins to assume a more compact character, its outlines are less irregular, and the capillary spaces decrease in numbers and size. (Fig.52, Pl.XII)

The nephrostome opens about the middle of the pronephric chamber. The tubule again loops anteriorly to the tip of the pronephric tissue before turning in a posterior direction. Its posteriorly directed loop splits into several branches which, after running a short distance at well-separated intervals through the renal tissue, turn dorsally and converge into the single pronephric duct. The latter is situated in the extreme dorsal tip of the pronephros, approximately opposite the mid-line of the notochord. This relative position will be maintained consistently in later stages.

The influence of the now well-developed air-bladder is clearly seen. The air bladder of the catfish sends out two long anterior sacs from the principal median, unpaired chamber. These sacs now crowd the renal tissue toward the median line, and the posterior tip of the pronephros narrows to a small wedge of renal tissue beneath the notochord, against the anterior tip of the main body of the air-bladder and compressed between its anteriorly extended sacs.

The pronephric ducts run between notochord and air-bladder, and at the same transverse level with the dorsal wall of the latter. The ducts are accompanied by the anlagen of the cardinal veins. The simple endothelium forming the walls of these vessels encloses a space filled with deeply staining plasma in which occur the developing blood corpuscles. (Fig.53 Pl.XII)

Ducts and blood vessels traverse the internephric space alone. They are not accompanied at this time by pseudolymphoid tissue, although this has been described by a number of authors as surrounding the "ureter". The heavy, muscular walls of the air-bladder press closely against the epithelium of the duct, which is often in contact with the sheath of the notochord.

A thin sheet of mesonephric tissue extends under (dorsal to) the posterior tip of the air bladder. Immediately behind the latter, the mesonephros broadens out into a heart-shaped organ of pseudolymphoid tissue traversed by mesonephric tubules.

Undifferentiated tubules are frequently encountered in cross-section. The cells of these are in contrast to the true epithelium of the duct, and may represent transitional stages. Nuclei and cytoplasm of these primitive tubules stain much more darkly than those of the adjacent ducts. The tubule cells, however, are wedge-shaped, and show the typical radial arrangement about a very small lumen. (Fig.31,Pl.IX)

The undifferentiated tubules appear to be short, coiled structures which end blindly. The definitive tubules show more ramifications than the tubules of the sixth day.

The most significant advance in development consists of the appearance of the Malpighian glomeruli of the mesonephros. (Fig.54,Pl.XII)

These glomeruli clearly project into the wall of a tubule, forming a Bowman's capsule, but although the opening of the tubule into the cavity of Bowman's capsule appears in many sections, it cannot

Fig. 31. Section through the mesonephric region of a larva of the seventh day. L.T., pseudolymphoid tissue; M.T., mesonephric tubule; M.T.A., mesonephric tubule anlage. 380 X

Fig. 32. Section through the pronephros of a sixth day larva. C.V., cardinal vein; L.T., pseudolymphoid tissue; P.T., pronephric tubule. 380 X

Fig. 33. Section through a degenerating tubule in the pronephros of a ninth day larva. L.T., pseudolymphoid tissue; P.T., pronephric tubule. 380 X

Fig. 34. Section through Bowman's capsule and glomerulus of a seventh day larva. B.C., Bowman's capsule; C.V., cardinal vein; G., glomerulus; L.T., pseudolymphoid tissue; M.T., mesonephric tubule. 380 X

Fig. 35. Section in the pronephric chamber region of an eleventh day larva. Gl. glomus; L.T., pseudolymphoid tissue; P.C., pronephric chamber; P.T., pronephric tubule. 380 X

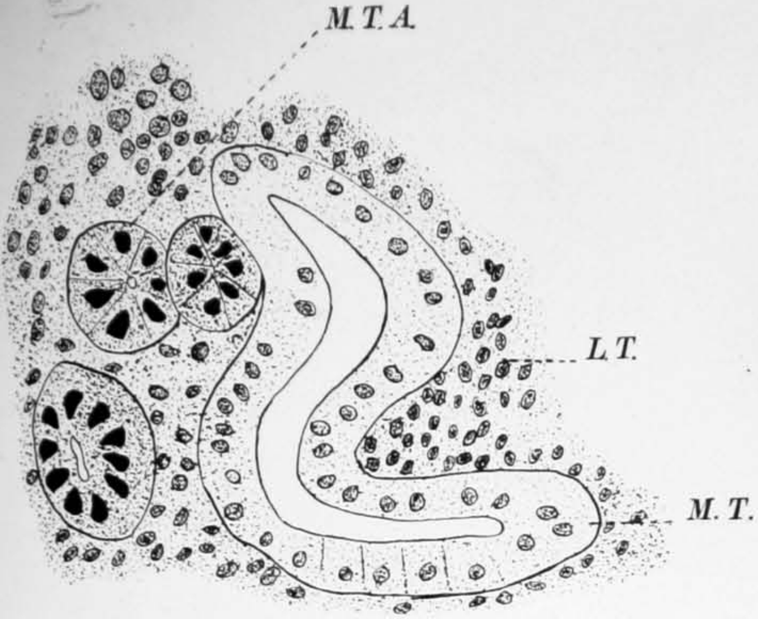


Fig. 31

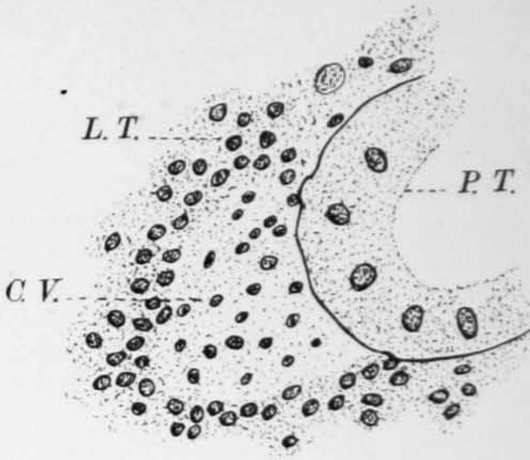


Fig. 32

Fig. 33

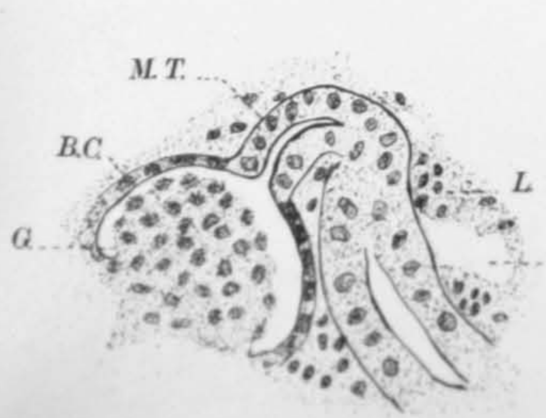


Fig. 34

Fig. 35

be determined positively from this material whether the cavity of the Malpighian corpuscle is continuous through the tubule with the lumen of the duct. In one case there are marked indications that this condition may occur. From the fact that in many instances the tubules with which the Bowman's capsule appears to connect show extremely small lumina, it may be that the glomeruli develop while the tubules are in a relatively undifferentiated state, and possibly are not yet in communication with the duct.

Bowman's capsule is lined by a flat epithelium. The glomerulus has the form of a somewhat flattened oval. The greater diameter of an average glomerulus of this stage is .0375 mn.; the lesser diameter, .0200 mu. (Fig.34,Pl. IX)

The Wolffian duct (the continuation of the pronephric duct, which now received mesonephric tubules,) leaves the kidney near its ventral margin. The two ducts run side by side above the intestine, imbedded in a small amount of pseudolymphoid tissue for a time.

The ducts elongate slightly in the dorso-ventral plane, fusing into the unpaired "urinary bladder". This bladder then runs posteriorly through eighteen sections before expanding into a roughly triangular shaped sinus, which sends a blind diverticulum anteriad ventral to the bladder through six sections. (A similar "blinder Fortsatz der Harnblase" was described by Hoffmann (1886).

While the sinus is still in the shape of an inverted Y, and remains a considerable distance dorsal to the intestine. (as shown in

Fig.43, Pl. X , for the ninth day, the intestine opens to the exterior through the anus. One prong of the Y is then retracted, the sinus elongates greatly, and runs ventrally, as in figures 44 and 45 Pl. X , for the ninth day. The urinary opening here cannot be determined, but its presence was established in the preceding and succeeding stages.

The eighth day

For the first time the pronephros shows definite relations with the venous system. The spaces of the posterior cardinal vein appear throughout the pseudolymphoid tissue. One large vessel on each side, elongated dorso-ventrally, is supplemented by numerous small capillaries. (Fig. 55 Pl. X)

The large glomus is situated medially ventral to the aorta and dorsal to the oesophagus, and presents no new features.

The single pronephric tubule sends many ramifications anteriorly and posteriorly through the pronephric tissue. Only one pronephric tubule is present, however, -a fact which is suggested by the presence of but one nephrostome on each side opening to the pronephric chamber, and which was verified by tracing the tubule through the sections. The amount of ramification is indicated by the fact that as many as six loops of the tubule are cut through in one section.

The lateral compression of the posterior end of the pronephros by the anterior horns of the air-bladder takes place as in the preceding stage. The pronephric duct as it leaves the anterior kidney occupies its usual position with reference to air-bladder and notochord.

The only new feature presented by the mesonephros is the increased development of the venous system here as in the pronephros.

The ninth, tenth and eleventh days

Few changes other than an increase in complexity of structures already present occur in larvae of the ninth, tenth and eleventh days. To avoid unnecessary repetition, therefore, these stages will be described together, and any significant differences will be noted.

The pronephric chamber remains a structure of considerable size on the ninth day. It occupies in its average condition, seven sections from anterior to posterior (section thickness = 10 mu.) The chamber at this time shows clearly its bilateral symmetry with respect to the thin median partition, from which the masses of glomar tissue are suspended.

On the tenth day, the chamber has become slightly shorter, occupying on the average five sections. (It is recognized that the number of specimens upon which these averages were computed is too small to render the figures significant in themselves. The condition found in the next stage, however, indicates that a decrease in the size of the chamber may be initiated at about the tenth day.)

From a clearly defined structure of relatively large size on the ninth day, the pronephric chamber has become reduced in one larva of the eleventh day to a mere remnant of its former extent. Several small spaces are discernible in the region normally occupied by the chamber. In one section, what is evidently the glomus (here a very indistinct mass), is separated from the adjacent tissues by a small slit-like space, which may be supposed to represent the vestige of the chamber. The occurrence of a tubule adjacent to the aperture lends support to this assumption. (Fig.35, Pl.IX) Very slight traces of the supposed chamber occur in five sections, - an area corresponding to that occupied by the chamber in the preceding stages.

In another eleven-day larva studied, the reduction of the chamber is less marked. Glomus and chamber are little less distinct than in earlier stages, although at the left ventro-lateral border of the chamber, the glomus is in contact with the wall of the chamber and is scarcely distinguishable from the pseudolymphoid tissue. The glomus and chamber are crowded out posteriorly on the left side by the tissue containing the mesenteric artery.

A study of additional material in this and succeeding stages is necessary before it can be stated positively that the apparent degeneration seen in one individual is actually the beginning of an atrophy of the pronephric chamber and its glomus which is characteristic of this stage, and not merely an individual difference.

The single pronephric tubule remains a much ramified structure on the ninth day. The point at which the segmental tubule appears to communicate with the duct occurs at the extreme anterior end of the pronephros, i.e., in the first section in which renal tissues appear. The duct lies dorsal to the loops of the tubule. As before, the pseudolymphoid tissue forms the substance in which the coils of the tubule are imbedded. The pronephric tubule of the tenth day also ramifies widely throughout the renal tissue. The large cardinal vein appears in intimate relation to the tubule, and may be traced anteriad after it leaves the pronephros as a blood-filled vessel of considerable size.

On the eleventh day, the pseudolymphoid tissue occupies a larger proportion of the pronephros than does the tubule.

Certain changes in the appearance of the tubule epithelium which are evident at this time may possibly represent the beginnings of cellular degeneration. The cell membrane adjacent to the lumen is frequently broken down, and the granular cytoplasm appears to extend into the lumen. (Fig.33 , Pl.IX) This phenomenon has been observed in the pronephric tubules of the ninth, tenth and eleventh days. The possibility that these apparent changes may have been the result of poor fixation has been considered, but the fact that mesonephric tubules in the same larvae show epithelial cells with clearly defined outlines indicates that the fixation is satisfactory.

The nephrostomes open close to the anterior tip of the pronephric chamber in the ten-day larvae. No nephrostomes could be distinguished in the next stage.

The position of the duct as it leaves the pronephros is determined by the encroachment of the cartilage surrounding the notochord upon the region formerly occupied by the duct. The latter lies somewhat ventral and lateral to its position in earlier stages.

The unbroken continuity of the duct in the oldest larvae studied is in doubt. In the long stretch dorsal to the air-bladder, between pronephros and mesonephros, the duct cannot be distinguished in all sections.

The various changes which have been described above, and which will now be recapitulated briefly, are thought to be of possible significance as indications that atrophy of the pronephros and its duct may be initiated in the rapidly developing catfish larvae about the tenth day after fertilization.

The pronephric chamber becomes smaller, its cavity in some cases being almost completely obliterated. The glomus loses the characteristics of a functional glomar organ which it exhibited during early larval life, when the pronephros may be supposed to have been the principal, if not the only functional excretory organ. The epithelial cells of the tubules show a breaking down of the cell walls, and the pseudolymphoid tissue increases in relative amount. The nephrostomes are indistinct, or apparently lacking. The lumen of the duct may not be continuous from pronephros to mesonephros.

Additional confirmation of these phenomena and a study of the fate of the pronephric structures in later stages is unquestionably necessary before a definite interpretation of the conditions described can be attempted.

The encroachment of the rapidly developing organs adjacent to the pronephros results in the compression of the renal tissue into a small space bounded laterally and caudally by the air bladder. The plastic substance of the pronephros assumes the shape of the cartilage against which it rests dorsally. The ducts as they pass posteriad are pressed against the notochordal sheath by the muscular wall of the air bladder.

No significant changes are evident in the mesonephros of the ninth day. A few relatively undifferentiated tubules appear. Venous spaces are numerous throughout the organ.

Near the periphery occur a small number of structures which closely resemble the anlagen interpreted tentatively as the corpuscles of Stannius. The migration of these structures toward the surface of the mesonephros is in accordance with the facts reported by Felix.

The shape of the whole organ in these larvae now approximates the adult condition. It is roughly triangular, the broad anterior end forming the base of the triangle, the caudal end the apex. The cranial region of the mesonephros retains a considerable amount of pseudolymphoid tissue. This element diminishes rapidly as the posterior end is approached. In the caudal half of the organ the tubules are so closely packed as to be almost contiguous, and the pseudolym-

Figs. 36-39. Sections through a sixth day larva in the region of the urinary bladder. I., intestine; U.A., urinary aperture; U.B., urinary bladder. 150 X

Figs. 40-47. Sections through the urinary bladder region of a ninth day larva. Lettering as above. An., anal opening; D., diverticulum of urinary bladder. M.D., mesonephric duct. 150 X

Plate X

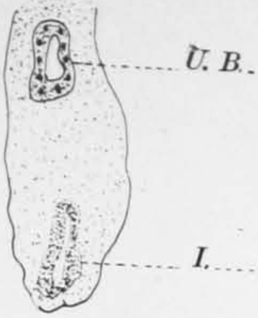


Fig. 36

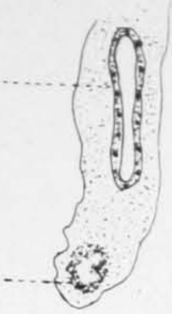


Fig. 37

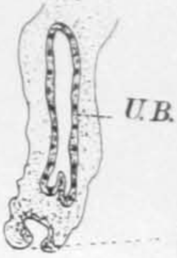


Fig. 38

Fig. 39

Fig. 40

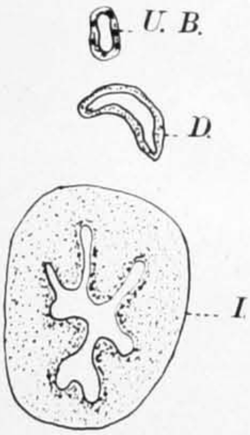


Fig. 41

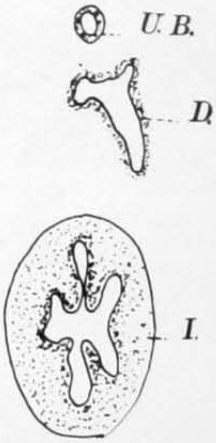


Fig. 42

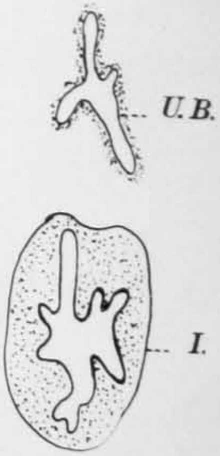


Fig. 43

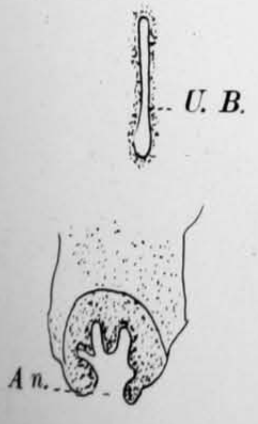


Fig. 44

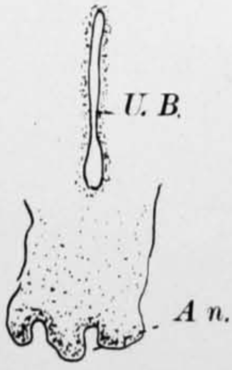


Fig. 45

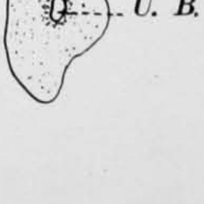


Fig. 46

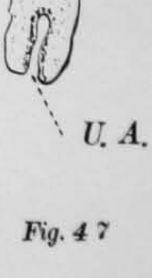


Fig. 47

phoid tissue is reduced to a minimum.

The Wolffian ducts open to the exterior in the manner described for preceding stages. The minute opening lies seven sections posterior to the anal opening. (Fig.40-47 Pl. X)

These relations are unaltered on the tenth day, except that a space of nine sections intervenes between the anus and the opening of the bladder. On the eleventh day an interval of twelve sections occurs. The urinary bladder is now large and thick walled.

DISCUSSION

Many of the theoretical considerations which are usually discussed in connection with the pronephros concern the early development of the organ.

The most primitive stages in the ontogeny of the excretory organs of Ictalurus were necessarily excluded from the present investigation, so that no direct evidence was obtained on these questions. The condition of certain structures in the earliest embryos studied, however, and their fate in succeeding stages, seems to show that some of these theories find confirmation in the catfish, while others are not substantiated. The question of the origin and significance of the pronephric chamber, and its possible homology with the Bowman's capsules of the mesonephros, are examples.

The anlage of the anterior end of the pronephros in two-day embryos appears at first to lend itself to any one of several interpretations. After a study of this and succeeding stages, it becomes apparent that one explanation has the balance of probability in its favor, and this has been adopted in the description of the embryos. The possible interpretations were:

1. The pronephric anlage has arisen from a simple isolation of the internal part of the splanchnocoele and its walls. Both somatic and splanchnic epithelium have intervened in its formation. By a process of rotation, the anlage has come to occupy its present position dorsal to the remainder of the splanchnocoele, so that it rests upon somatic epithelium. Its continuity with the cavity of the splanchnocoele is maintained, but the position of the opening has been altered in conformity with the rotation, so that it now occurs in the dorso-lateral wall of the body cavity. This account is in conformity with the description of Swaen and Brachet, and is true in general for all forms described by them.

2. The anlage seen on the second day has been preceded by a stage (as described by Felix for the trout) where a series of pronephric tubules project from the splanchnocoele toward the ectoderm. The tubules fuse into a continuous mass, the pronephric fold. The combined nephrostomes of the tubules are represented by the opening from the splanchnocoele into the pronephric fold, and should thus be designated a nephrostome. The external end of the fold (which has arisen by the fusion of the ends of the tubules) constitutes a collecting duct.

If the anlage of the pronephros arose from a series of tubules which subsequently fused into a continuous fold, there is certainly no trace of this originally segmented condition in the youngest embryos of Ictalurus studied. This fact obviously does not constitute proof that no such segmentation has preceded the two-day stage, but the contrary evidence of Swaen and Brachet and their plausible suggestion as to the source of the observational error of Felix cannot be disregarded.

There seems to be no reason to question the accuracy of the painstaking account of Swaen and Brachet. Their investigation covered a wide range of Teleosts, and it will be recalled that while the trout presented certain unique features, all other forms studied by them were almost identical in their mode of development. The members of the second group, however, resemble Ictalurus in the small size of their eggs and the fact that they develop with great rapidity, hatching in about five days. The differentiation of the pronephric chamber of Ictalurus after the second day corresponds so closely to that of the forms described that it appears possible that its earlier ontogeny is also similar.

Whether the pronephros of Ictalurus arose from a series of segmentally arranged pronephric tubules, or whether, as seems more probable, it represents the isolated internal angle of the splanchnocoele and its walls, its later development agrees with the accounts of Felix and of Swaen and Brachet, which are similar for the later stages.

The internal region of the anlage becomes expanded into a rounded vesicle. Its lining epithelium is transformed into the flattened type. The outer end of the anlage, the cuboidal epithelium of which is unchanged, remains an elongated structure with a narrow lumen, and communicates with the expanded pronephric chamber through a nephrostome. The lateral portion of the anlage is interpreted as the single pronephric tubule. It communicates caudally with the pronephric duct, which it is assumed has been developed in the same manner as the anterior end of the anlage, except that it has already undergone complete separation from the splanchnocoele. The anterior part of the pronephric anlage migrates toward the median line above the general body cavity, losing at the same time its primitive connection with the latter. Vascular relations are established through the formation of a glomus in the mesoderm medial to the chamber. Pronephric chambers of the right and left sides are ultimately separated by only a thin median septum. The glomus comes to project into, and almost fill, the cavity of the chamber.

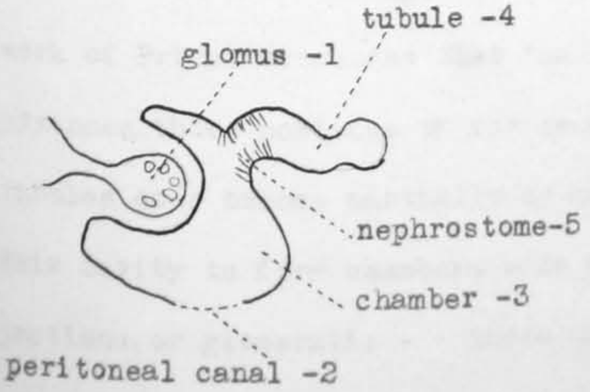
The structure thus formed, consisting of glomus and pronephric chamber, is often referred to in the literature as a "Malpighian corpuscle", the authors apparently recognizing no distinction in origin or structure between the glomerular organs of the pronephros and mesonephros. It is, in fact, not uncommon to encounter the statement that the two are homologous structures.

Audigé (1910) states that the pronephros contains "deux volumineux glomérules de Malpighi" which "sont enveloppés d'une capsule de Bowman (persistence de la chambre pronéphrotique embryonnaire").

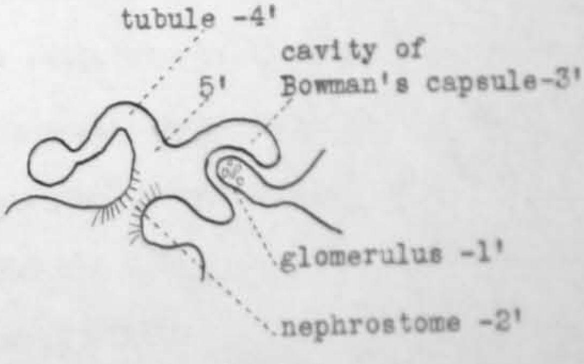
Emery (1880) and Swaen and Brachet (1902) use the term "Malpighian corpuscle" without explanation or comment.

Price (1897) believes that the coelomic pockets in Bdellostoma are homologous with the pronephric chambers of the Teleostei. The whole excretory organ is thought to arise as a pronephros, the posterior region subsequently undergoing differentiation as a mesonephros. Price concludes that there is no essential difference in the manner of formation of pronephric and mesonephric tubules. The coelomic pockets occur at segmentally arranged intervals along the whole length of the duct by the fusion of the somatic and splanchnic epithelium between the opening of the tubule and the main cavity of the splanchnocoele. The isolation of this inner angle of the body cavity which receives the pronephric tubule unquestionably suggests the development of the pronephric chambers of Teleosts. Price further states, however, that the coelomic pockets form an important part of the glomerular structure of the mesonephros. The cavity of the pocket becomes the cavity of a Malpighian corpuscle. The glomerulus projects against the inner angle of the wall of the tubule, and therefore is covered partly by the epithelium of the latter, and partly by somatic epithelium. Bowman's capsule itself is composed of the splanchnic epithelium which constitutes the floor of the pocket. If, as Price's observations led him to state, pronephric and mesonephric tubules have a similar origin, one would expect no difference in the development of their glomerular structures. The case of Bdellostoma, however, does not appear to be typical of all Anamniota.

In order to homologize the pronephric glomus and its chamber with the Malpighian corpuscle of the mesonephros, one of two postulations must be accepted. Either the pronephric chamber into which the glomus projects must have been derived from the pronephric tubule or the cavity of Bowman's capsule must represent an isolated part of the splanchnocoele. In either case the cavity of the Malpighian corpuscle would be homologous with the pronephric chamber. The two structures might then be compared part for part: the "peritoneal canal" from the pronephric chamber to the splanchnocoele with the nephrostome; the glomus of the pronephros with the glomerulus of the mesonephros; the pronephric chamber with the cavity of Bowman's capsule. The "nephrostome" opening into the pronephric chamber would then correspond to the portion of the mesonephric tubule which communicates with the cavity of Bowman's capsule.



Pronephric chamber



Malpighian body

The accuracy of neither of the basic premises upon which the homology rests is beyond question. Although Felix infers that the pronephric chamber is derived from tubules, and his interpretation is generally accepted, there is, so far as is known, no actual evidence other than that of Felix which supports this theory. Even if it be accepted that the whole pronephric anlage represents one or more tubules, the fact remains that the tubules themselves which gave rise to the chamber were derived from the splanchnocoele and its walls. Unless Bowman's capsule also represents an isolated part of the splanchnocoele, it is difficult to see how it can be homologized with the pronephric chamber.

Although Price (1897) emphasizes the similar development of pronephric and mesonephric tubules in Bdellostoma, and states that the coelomic pockets which occur throughout the whole excretory organ are probably homologous with the pronephric chambers of Teleosts, he denies in a later paper (1905) that he wishes to imply any homology between the glomeruli which are associated with the coelomic pockets and the glomi of higher forms.

Burlend (1931), however, commenting in a recent paper on the work of Price, concludes that "as the evolution of the nephric system advances those portions of the splanchnocoele into which the nephric tubules open become partially or completely separated from the rest of this cavity to form chambers with which are associated vascular projections or glomeruli; - - these chambers are doubtless the homologues of the Bowman's capsules found in the later evolved meso- and metanephros."

Burlend seems clearly to infer that Bowman's capsule represents an isolated portion of the splanchnocoele. The whole nephric system of the Anamniota, according to Burlend's interpretation, arises from a primitive nephric groove which became differentiated from the somatopleuric mesoderm. The medial portion of the groove is represented in the anterior region by the pronephric tubules and in the posterior by scattered cells which later become arranged to form the mesonephric tubules. The fact that the mesonephric tubules often arise from the nephrotome is accounted for by the evolutionary change through which the primitive nephrotome is supplemented by an addition derived from the wall of the primitive splanchnocoele. With this change is associated the arrested development of the mesonephric tubules. If Burlend's interpretation of the evidence is correct, then a part of the splanchnocoele is actually included in the mesonephric tubule, and this might very probably constitute the cavity of Bowman's capsule.

The account of Felix, which has already been mentioned, is directly at variance with this theory. This author does not recognize the origin of the mesonephric tubule anlagen from the primitive nephrotome, but states clearly that the antecedents of the cell masses which give rise to the tubules are unknown, although he thinks they may have developed from cells in contact with the peritoneal epithelium. Other authors who support this view have already been quoted.

The whole question appears to rest upon speculations and inconclusive evidence. In the absence, therefore, of a well-established foundation for the homology, it seems desirable to avoid the use of the term "Malpighian corpuscle" to represent the pronephric chamber and glomus.

The condition found in the catfish demonstrates that the pronephric chamber in its primitive condition is in open communication with the general body cavity. The glomus comes to project into this chamber, i.e., into an isolated part of the splanchnocoele. The mesonephric tubules appear to arise from solid groups of cells of uncertain origin which occur in the mesoderm adjacent to the pronephric duct. The lumen and the cavity of the true Malpighian corpuscle are developed secondarily.

The sudden appearance of the so-called lymphoid tissue ("pseudolymphoid tissue" of Felix) in the pronephros and mesonephros of Ictalurus larvae at the time of hatching has been noted elsewhere. The possible relation of this tissue to the developing circulatory system is worthy of more detailed consideration.

The pseudolymphoid tissue, in its definitive state, does not appear until all the purely renal elements of the pronephros have been laid down and have undergone considerable differentiation. On the sixth day after fertilization, it appears suddenly in both the pronephric and mesonephric regions, although it is more abundant in the former. Here its topographical relation to the developing cardinal veins is apparent.

The embryology of the circulatory system is not within the scope of the present investigation, but the salient features of the development of the vascular tissue in relation to the excretory organs have been noted. A tissue of striking appearance makes its appearance on the fourth day, adjacent to the anterior end of the pronephric duct. It consists of a finely granular matrix in which are imbedded deeply-staining cells. In sections treated with Mayer's Haem-alum and Eosin, the matrix stains a pale pink. This tissue appears as a thin strand extending dorso-mesiad from the duct. It occurs more frequently in relation to the right duct, and always at this stage adjacent to either its dorsal or medial surface. The aorta in most sections appears as a fairly distinct vessel with endothelial walls. Immediately ventral to the aorta is a small mass of tissue similar to that which occurs in relation to the ducts.

The account of Brachet (1921) confirms the interpretation of this tissue as the beginnings of the circulatory system, corresponding in a general way to the blood islands of many Vertebrates. Brachet says of the "îlots sanguins": "Les Téléostéens diffèrent de tous les autres Vertébrés par la localization dans la région dorsale du corps de l'embryon, de ce qui represent chez eux les îlots sanguins". The vascular zone is described as consisting of a thin layer of cells which slips under the scleromyotome toward the median line, where it unites with the similar structure from the opposite side to form a solid axial rod. A liquid plasma soon becomes dissociated from the cells, which round up and assume the characteristics of primary erythrocytes. Immediately before this occurs, however, the more dorsal of the cells composing the axial mass arrange themselves about a central cavity as an endothelium. The vessel thus formed is the aorta. The axial cord persists for some time under the aorta, with no change except an increase in the amount of plasma. Little by little the more superficial of its cells become flattened and constitute an endothelial layer about the ventral portion of the original axial strand. The "axial vein" is thus formed.

"Les Masses intermédiares," Brachet concludes, "sont donc les ébauches communes de l'aorte, de la veine axial et de tous les erythrocytes qui se forment dans le corps de l'embryon; sa partie moyenne et ventrale n'est, par conséquent, qu'un long îlot sanguin."

In Ictalurus embryos of the fourth and fifth days, large masses of vascular tissue occur lateral to the pronephric chamber and in proximity to the tubule. (Fig.27, Pl.VIII) They accompany the duct in its posterior course toward the cloaca. In the anterior end of the duct they occur in relation to its dorsal surface. Here their diameter may be equal to, or greater than, that of the duct. Posteriorly, the vascular anlagen may occur in any position with respect to the duct. A single axial strand is ultimately formed between the ducts. This wedge of vascular tissue separates the ducts throughout the remainder of their course to the cloacal region. (Fig. 10, Pl. IV)

On the sixth day, the pseudolymphoid tissue appears in the pronephros in a position adjacent to that occupied by the undifferentiated vascular tissue on the fifth day. The whole structure, which may now be termed the pronephros, consists of a mass of pseudolymphoid tissue above the coiled tubule, and a larger amount of vascular tissue below. Lateral to the tubule, pseudolymphoid and vascular elements are freely intermingled. (Fig. 27, Pl. VIII) The cardinal vein may at this time be easily distinguished in the anterior pronephric region from the undifferentiated part of the blood island. It is filled with a light plasma and contains numerous cells with dark nuclei.

In sections containing the pronephric chamber, strands of vascular tissue are scattered throughout the pseudolymphoid elements. (Fig. 32, Pl. IX) These appear to be in a relatively high state of differentiation. The only apparent difference between the pseudolymphoid cells and those in the vascular areas is the larger size of the former and the fact that the developing blood corpuscles are scattered throughout the plasma, instead of being closely massed. The nuclei of both types of cells appear dark.

It is unknown whether the vascular tissue in the time intervening between the fifth and sixth days came to occupy the whole area which it now shares with the pseudolymphoid tissue, or whether the latter merely develops in the mesenchyme adjacent to the cardinal vein anlage. If the former possibility should prove correct, one might be led to share the belief of Felix that the pseudolymphoid tissue is proliferated from the walls of the cardinal veins.

According to Felix (1897), not all the peripheral cells of the unpaired "Venenstrang" are used to build up the walls of the cardinal veins. The "pseudolymphoid tissue" arises through a proliferation of both the endothelial cells of the venous wall and the accumulated cells around it. Thus is formed a framework in the meshes of which lie the veins and the glandular structures of the kidney. The cells of this supporting structure subsequently divide into two groups: those with dark and homogenous nuclei (the true pseudolymphoid cells), and those with clear nuclei in which a rich chromatin network occurs. The latter are designated "epitheloiden Zellen", and are believed by Felix to give rise to the blood corpuscles.

The rapid development of the catfish in contrast to that of the trout and salmon might logically account for differences in the details of the above descriptions. It is not the intention of the author to offer at this time any theory of the origin of the pseudolymphoid tissue in the catfish. The isolated facts at hand might easily lead to a wholly erroneous conclusion. Pending a more extensive investigation of the question, however, it is considered worthy of notice that the pseudolymphoid tissue occurs in close proximity to the undifferentiated tissue of the cardinal veins, appearing simultaneously in both pronephros and mesonephros.

SUMMARY

1. The anlage of the pronephros in embryos of the second day consists of an anterior region, the pronephric chamber, at right angles to the long axis of the embryo, and continuous posteriorly with a pronephric duct. The pronephric chamber retains its primitive connection with the splanchnocoele through a peritoneal funnel at its ventro-medial border. It communicates with the pronephric duct through a single pronephric tubule. The excretory system is not in communication with the exterior at this time.

2. The pronephric chamber undergoes differentiation into a medial expanded vesicle from the lateral margin of which the segmental tubule of the pronephros opens through a nephrostome. The glomus develops in the mesoderm between the pronephric chambers of the right and left sides. It pushes against the medial or dorso-medial wall of the chamber, forming an evagination into the cavity of the latter. The dorsal and ventral "horns" of the chamber lie above and below the glomus, and approach each other in the median line. This condition occurs in embryos of approximately seventy-two hours. The peritoneal canal at this time is closed, so that the pronephric chamber is completely isolated from the splanchno-

coele. The pronephric duct appears to communicate with the exterior in the cloacal region.

3. Pronephric chambers of the right and left sides come to lie in the median line, separated only by a thin septum. The originally distinct glomi are more or less completely fused into a single median mass, suspended from the septum. The nephrostome of the pronephric tubule opens into the chamber opposite the glomus. The pronephros of the fourth and fifth days is characterized by these features.

4. On the sixth day, the final component of the pronephros, the pseudolymphoid tissue, suddenly makes its appearance. It forms the principal substance of the large organ known as the pronephros, and the true renal elements are imbedded in it. The pseudolymphoid tissue also surrounds the mesonephric tubules.

5. Solid anlagen containing numerous nuclei occur in contact with the posterior, or mesonephric region of the pronephric duct on the fourth day. These, during the course of later development, become detached from the duct and migrate, as solid masses, to the periphery of the mesonephros. Anlagen of apparently identical characteristics are described in the literature as occurring in the trout and salmon, and are interpreted tentatively as representing the cortical portion of the supra-renal gland (Corpuscles of Stannius).

6. Simple mesonephric tubules in open communication with the Wolffian duct occur on the sixth day. Their exact origin has not

been determined, but a few undifferentiated tubules which occur in the same stage indicate that the definitive tubule arises from a solid mass of cells which become disposed about a central lumen. The latter acquires communication with the Wolffian duct only secondarily. Malpighian glomeruli appear on the seventh day.

7. The pronephric tubule becomes a much-coiled structure which traverses the whole pronephros. The vascular tissue, which is the fore-runner of the cardinal veins in an undifferentiated state, has occurred in proximity to the tubule at least since the fourth day. The definitive cardinal veins appear clearly in relation to the pronephric tubule of the eighth day, where their ramifying vessels occupy a considerable proportion of the pronephros.

8. The caudal ends of the pronephric ducts fuse into an unpaired urinary bladder. In early stages, the intestine ends blindly against the anterior wall of the bladder just dorsal to the opening of the latter to the exterior. The intestine subsequently acquires an anal opening anterior to the urinary aperture. With further development the openings are carried farther apart.

9. About the fourth day of larval life (the tenth day after fertilization) certain phenomena indicate that degenerative changes may have already been initiated in the pronephric region. The pseudolymphoid tissue increases at the expense of the tubule; the epithelium of the latter loses its distinct cellular character; the nephrostomes appear to be lacking in some cases; the pronephric chamber

and glomus are reduced in size and lose the characteristics which distinguished them during the period when the pronephros may be supposed to have attained its maximum physiological efficiency. The lumen of the pronephric duct appears to be obliterated in certain regions.

10. The encroachment of other structures, notably the air-bladder and the cartilage of the developing cervical vertebrae, has confined the pronephros within a small space anterior to the air-bladder. Its further development would thus seem to be effectively inhibited. The mesonephric tissue, which encounters no such obstacle to development, is meanwhile increasing rapidly in size and complexity.

Fig. 48. Section through the anlage of the pronephric chamber of a two-day embryo. Ch. an., chamber anlage; co., coelom; so. ep., somatic epithelium; spl. ep., splanchnic epithelium. 450 X

Fig. 49. Section through the glomus of a four-day embryo. Co., coelom; d.m., dorsal mesentery; gl., glomus; ne., nephrostome; pr.ch., pronephric chamber. 730 X

Fig. 50. Section through the pronephric ducts and the developing cardinal veins on the seventh day. c.v.an., anlage of cardinal veins; int., intestime; pr.d, pronephric duct. 450 X

Fig. 51. Section showing the coils of the pronephric tubule imbedded in pseudolymphoid tissue on the sixth day. Co., coelom; l.t., pseudolymphoid tissue; pr.t., pronephric tubule. 450 X

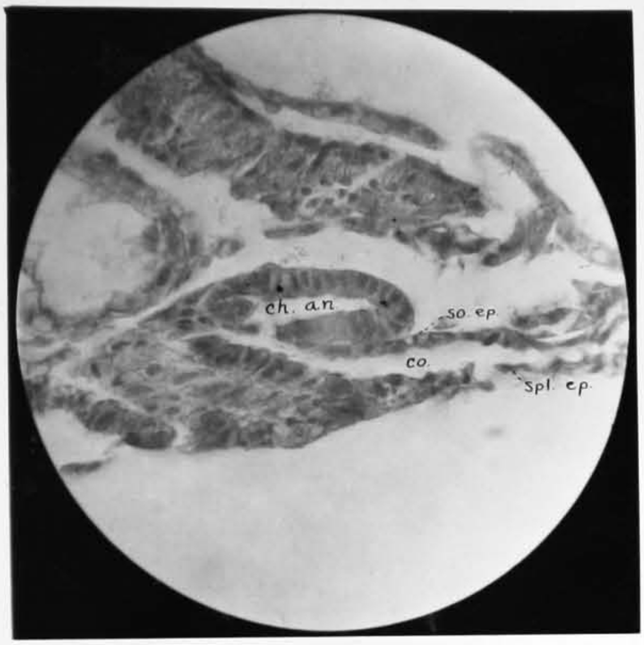


Fig. 48

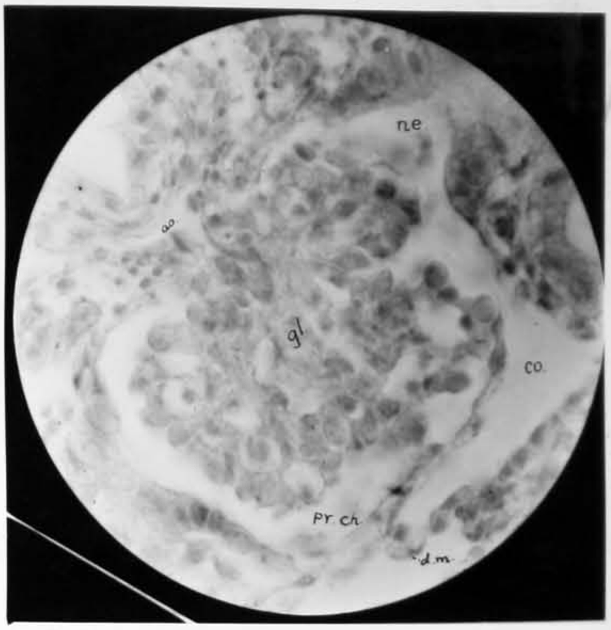


Fig. 49

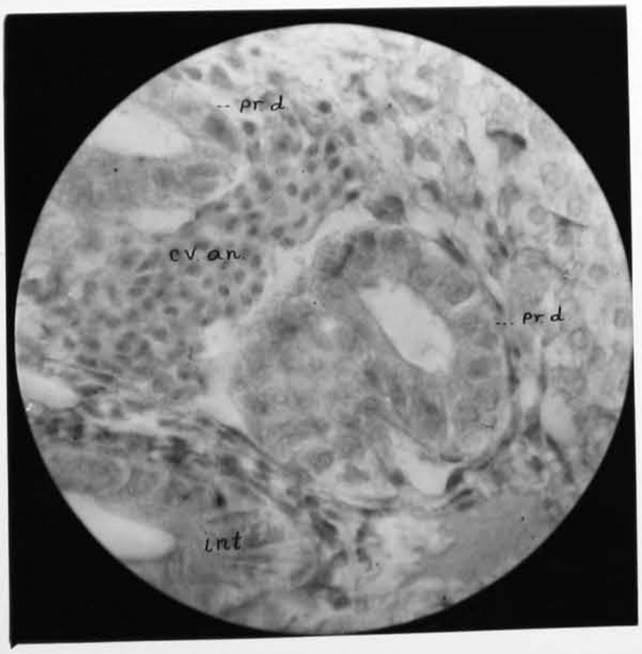


Fig. 50

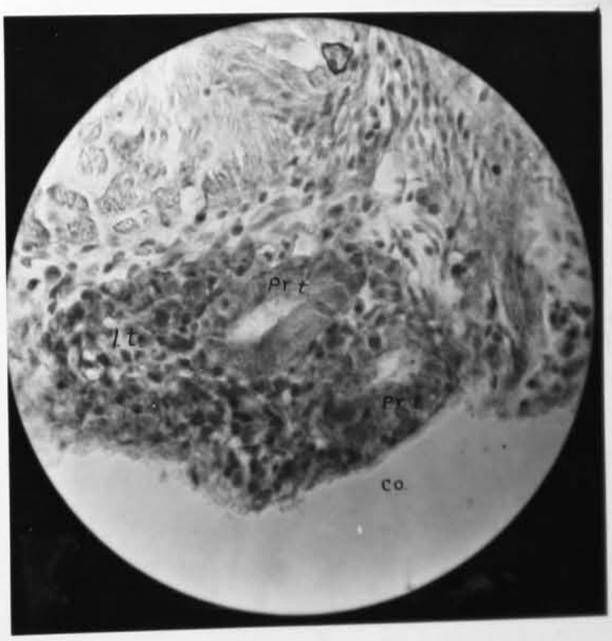


Fig. 51

Fig. 52. Section through the pronephros on the seventh day, showing the topographical relation of the glomus to the aorta. Ao., aorta; Co., coelom; gl., glomus; n., notochord; pr, ch., pronephric chamber. 450 X

Fig. 53. Highly magnified section through the pronephric duct and the cardinal vein in a seven-day larva. C.v., cardinal vein; pr.d., pronephric duct. 730 X

Fig. 54. Section through the mesonephros of a seven day larva. Co., coelom; g., glomerulus; mes.t., mesonephric tubule; n., notochord. 450 X

Fig. 55. Section through the pronephros on the eighth day, Co., coelom; gl., glomus; n., notochord; oe., oesophagus; p.c.v., posterior cardinal vein; pr.t., pronephric tubule. 85 X

PLATE XII

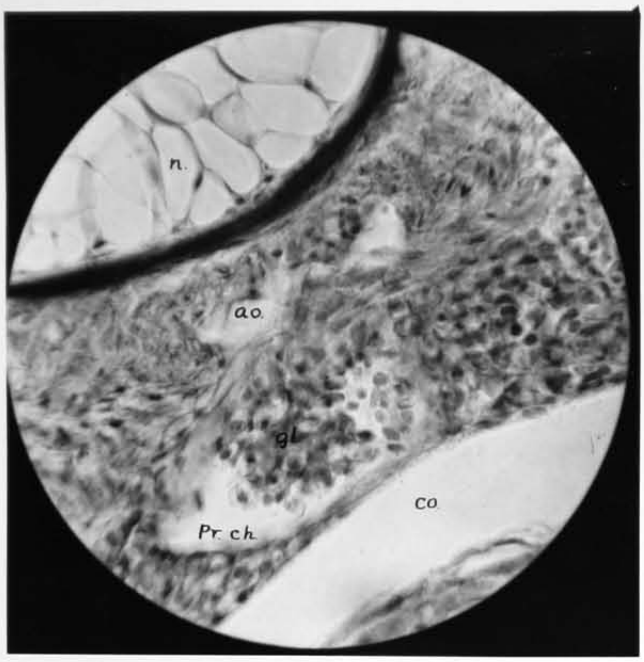


Fig. 52

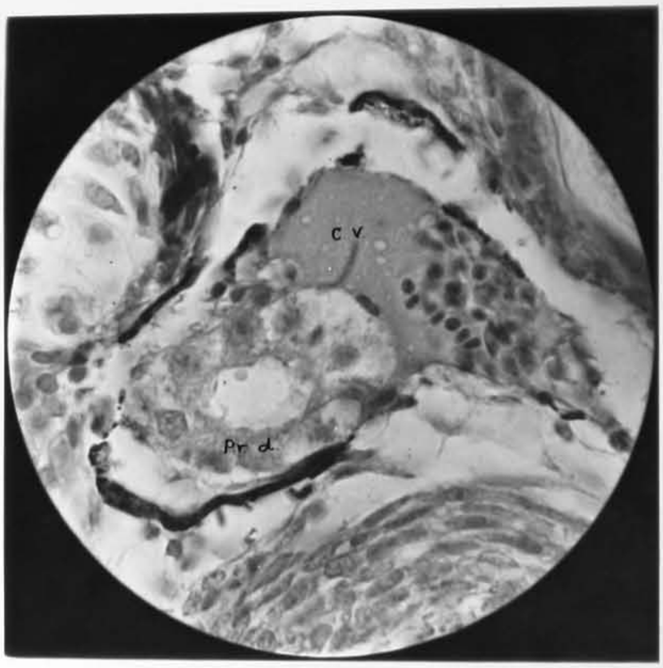


Fig. 53

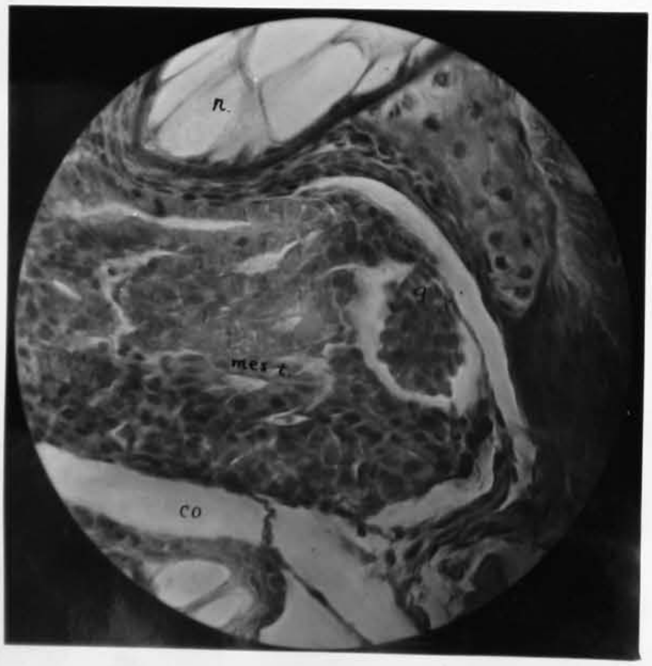


Fig. 54

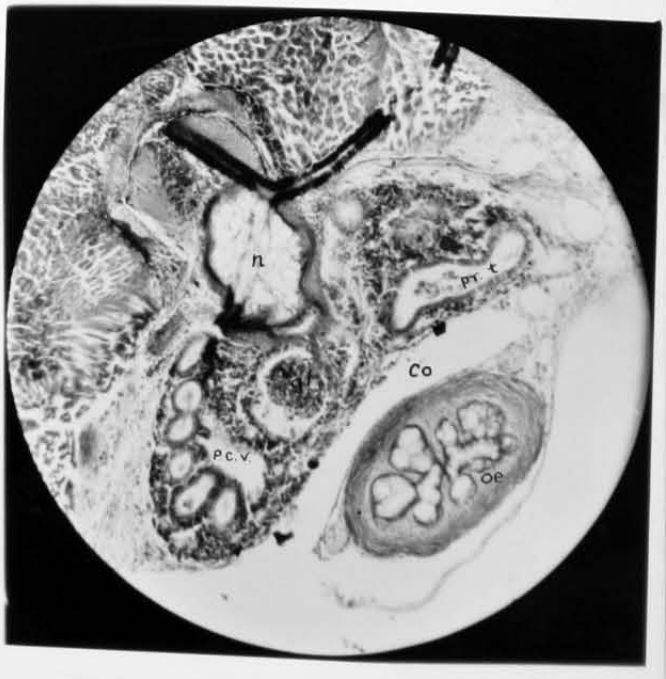


Fig. 55

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